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A SHORT TREATISE

ON

ANTI-TYPHOID INOCULATION.

CONTAINING

An Exposition of the Principles of the Method,

AND

A Summary of the Results achieved by its Application.

BY

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ANTI-TYPHOID INOCULATION.

PREFACE.

I am by the courtesy of the proprietors of The Practitioner permitted to republish in book form, with amplifications, a series of three papers on Anti-Typhoid Inoculation recently contributed to that Journal.

I endeavour to give here a brief exposition of the scientific principles involved in anti-typhoid inoculation and a summary of the practical results already achieved.

In Chapter I. I deal with the General Principles of Immunisation; in Chapter II. with the Application of these Principles to the particular Problem of producing Immunity to Typhoid Fever; in Chapter III. with the Technique and Clinical Effects of Anti-Typhoid Inoculation; in Chapter IV. with the Practical Results achieved as disclosed in the Statistical Records.

In connection with these Records I have had to consider the general questions which arise in connection with the cogency of statistical evidence.

All these subject-matters—not excepting that which is concerned with the appraisement of the statistics of anti-typhoid inoculation—make, it is to be feared, certain demands upon the reader in the form of intellectual effort.

For the circumstance that intellectual effort is called for in connection with the apprehension of a difficult and intricate subject-matter I would fain not be held responsible.

I may perhaps claim for the process of anti-typhoid inoculation that—first among the preventive inoculations which have application to man—it has been followed up step by step by scientific research.

The curves of immunisation which I have plotted out in the case of men inoculated with anti-typhoid vaccine, and the similar curves I have recently plotted out in connection with therapeutic inoculations of staphylococcus vaccine and tubercle vaccine respectively, are the outcome of a new system of technique devised for the measurement of the content in protective elements of small samples of blood withdrawn from the finger.

In conclusion, it may be well to make clear the following points :—

1. My work in connection with anti-typhoid inoculation in the Army—and it has included, in addition to laborious experimentation, the inoculation of 4,000 British soldiers all over India, and the supply of some 400,000 doses of anti-typhoid vaccine during the South African War—has throughout been gratuitously given.

2. Anti-typhoid inoculation was suspended in the Army 18 months ago at the instance of Mr. Brodrick's newly-created Medical Advisory Board.

3. The Royal College of Physicians, to whose arbitrament the question of the efficacy of the anti-

typhoid inoculations was referred by the Secretary of State for War, reported to him on July 27, 1903, in the following terms:—"After careful scrutiny of the "statistics from both official and private sources "which have been made available, we are of opinion "that not only is a lessened susceptibility to the "disease brought about as a result of the inoculations, "but that the case mortality is largely reduced."

4. A committee has recently been formed—and of this committee I have the honour to be a Member—to take up again in connection with the Army the consideration of anti-typhoid inoculation.

7, Lower Seymour Street, W.

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CHAPTER I.

ON THE PRINCIPLES OF PROTECTIVE INOCULATION AND ON THE PHYSIOLOGY OF IMMUNISATION.

THE problem of conferring protection against typhoid fever by preventive inoculation is in reality a physiological problem. It is the problem as to how to call into action, and how to direct towards the end that we have in view, the physiological machinery which is concerned with immunisation.

To do this we must learn all that we can with regard to the nature and range of operation of the physiological machinery in question, and all that we can with regard to the laws which govern its functioning.

Inasmuch as the existence of the faculty of immunisation is as yet altogether unrecognised by the physiologist, it may at the commencement be well to set before ourselves the evidence which establishes the existence of such a faculty as a physiological entity.

That evidence is furnished on the one hand by clinical observation, and on the other hand by immunisation experiments undertaken in the laboratory.

The former, testifying as it does, on the one hand, to a spontaneous recovery from bacterial infections, and, on the other hand, to the acquirement by the convalescent of insusceptibility to further infection, furnishes, even taken by itself, adequate evidence of the organism is furnished with a machinery for immunisation.

The efficiency of the machinery is attested—

1. By the fact that it is now everywhere felt that our proper course in the presence of specific fevers of unknown causation is to stand aside and allow the organism to develop its own defences.

2. By the fact that the methods of treating tubercular disease by the internal administration of antiseptics have been practically everywhere laid aside in favour of methods which are, at least in intention, methods for building up the constitution, and for the increase of its defensive powers.

3. By the fact that it is now recognised that where protective substances obtained from animals vicariously inoculated are available, these supply the most effective agency for combating bacterial infections.

MACHINERY OF IMMUNISATION A CHEMICAL MACHINERY.

In embarking upon the consideration of the physiology of immunisation, we may take our departure from the general proposition that the process of immunisation is in every case associated with an elaboration of chemical products on the part of the inoculated organism. Even where, as in the case of anti-staphylococcic inoculations, the condition of immunity which is achieved appears at first sight to depend upon an exaltation of the power of the phagocytes, it is, as I have shown[1] in conjunction with Captain Stewart Douglas, I.M.S., in reality referable to the appearance in the serum of a special class of protective substances—*opsōnins*.

The machinery of immunisation must, in conformity with the above generalisation, be conceived of as a purely chemical machinery. It is a machinery which elaborates, in response to the appropriate chemical stimulus, the particular internal secretion which is demanded for the purposes of immunisation.

I cannot, without losing touch with the practical end which I have here in view, pause to consider the speculations of Ehrlich with regard to the pattern and design of this chemical mechanism. Valuable, and indeed priceless to the intellect as the conceptions in question are, we shall do well here to turn aside from them to consider the practical question of the relation which obtains between the chemical element which sets in motion the machinery of immunisation, and the product which is elaborated by that machinery.

CHEMICAL RELATION BETWEEN THE SUBSTANCE INOCULATED AND THE PRODUCT WHICH IS ELABORATED IN THE ORGANISM IN RESPONSE TO THAT INOCULATION.

The relation between the foreign element which is inoculated—we may conveniently speak of this, irrespectively of its derivation, as the *vaccine*—and the chemical product which

[1] *Proc. Royal Society*, October, 1903, and February, 1904.

makes its appearance in the blood of the inoculated animal was first clearly discerned by Ehrlich. The correctness of the conception which he formed of this relation was first demonstrated by him in connection with his experiments on ricin.

The chemical properties of the vaccine inoculated and the product of immunity obtained were in these experiments as follows:—The substance inoculated was, as has already been indicated, ricin, a chemical principle derived from castor-oil seeds. Ricin added to blood, whether *in vivo* or *in vitro*, exerts an agglutinating and finally a disintegrating effect upon the red blood-corpuscles. The vaccine employed was thus, in the convenient technical language of Ehrlich, a "hæmotropic" poison —a poison which enters into chemical combination with the blood. Experiments with the blood of animals immunised by the inoculation of progressive doses of the poison showed (*a*) that the red blood-corpuscles of such blood were quite unaffected by an addition of ricin; (*b*) that the red blood-corpuscles of normal blood were unaffected when mixed with a sufficiency of the serum of the immunised animal, and (*c*) that ricin was deprived of all its poisonous properties by digestion with the serum of an immunised animal.

It was thus made manifest that the serum of an animal which has been immunised by progressive doses of ricin must contain an element which combines with the ricin, intruding itself between the poison and the constituent of the blood, upon which it is accustomed to fasten. We may speak of this product of immunity, which has appeared in the blood, as a "ricinotropic element."

The data obtained by Ehrlich in this investigation were soon supplemented by further data. In particular it was established by the work of C. J. Martin[1] that the chemical element which is obtained in response to the inoculation of snake-venom is an element which combines with that venom—a "venenotropic element;" and further, that the chemical element obtained in response to the inoculation of diphtheria-toxin is an element which combines with that poison—a "toxitropic element."

It was soon afterwards discovered that the subcutaneous or intravascular inoculation of blood from an animal of different

[1] *Proc. Royal Society*, Vol. 63.

species is responded to on the part of the inoculated animal by the elaboration of hæmotropic elements—in particular, of hæmolysins. It was then discovered that the subcutaneous inoculation of milk is responded to by the elaboration of a galactotropic element—in particular, of an element which confers upon the blood a power of coagulating milk. Later again, it was ascertained that the inoculation of testicular extracts and ciliated epithelium is responded to by the formation of spermatotropic and epitheliotropic substances which possess the power of arresting the movements of spermatozoa and epithelial cilia respectively.

It had already been ascertained—and it concerns us to bring this into relation with the above facts—that the inoculation of bacterial elements is responded to by an elaboration of bacteriotropic elements. Under this name we may group together the various substances which produce, as the case may be, agglutinating, bactericidal, bacteriolytic, or, as already indicated, opsonic effects.

We are manifestly led up by all these facts to the generalisation that in every case the inoculation of a vaccine is responded to by the elaboration of a vaccinotropic element. It will be convenient—though this will involve a certain departure from Ehrlich's system of nomenclature—to speak of the products of immunity generally as *antitropic elements*, or more briefly, *antitropins*.

SPECIFICITY OF THE ANTITROPIC ELEMENTS WHICH ARE PRODUCED IN THE CASE OF EACH PARTICULAR INOCULATION.

The next point to be insisted on is the specificity of the antitropic elements which are produced in response to different vaccines. The hæmotropic, bacteriotropic, and other antitropic substances spoken of above, exert in each case a specific action upon the chemical substances which have been employed as vaccines. A hæmotropic element obtained by the inoculation of a particular variety of blood is restricted in its action to the blood of the animal which has furnished the vaccine, or to the blood of an animal closely related to the same. In a similar manner, a bacteriotropic substance obtained by the inoculation of a particular micro-organism is operative only

upon the particular species of micro-organism in question. A tubercle-vaccine, for instance, is responded to, so far as it is known, only by an elaboration of tuberculo-tropic elements; a typhoid vaccine only by elaboration of typhotropic elements; a staphylococcic vaccine only by elaboration of staphylotropic elements.

CHARACTER OF THE ANTITROPIC ELEMENTS OBTAINED IN THE CASE WHERE THE VACCINE WHICH IS EMPLOYED CONTAINS IN ADDITION TO SUBSTANCES HELD IN SOLUTION ALSO FORMED ELEMENTS.

In the case of inoculations undertaken upon an animal with the blood of another animal, we are dealing with a vaccine such as is here contemplated. We are in such a case inoculating red and white blood-corpuscles suspended in a plasma or serum which contains a variety of albuminous substances. In the case where a bacterial culture is inoculated, we are similarly dealing with formed elements suspended in a solution which may contain a variety of other bacterial products. It will be instructive to consider what will happen in these cases. When we inoculate blood, we obtain as our harvest a complicated mixture of hæmotropic elements, in particular erythrocytotropic elements, leucocytotropic elements, and elements which we may group together as serotropic elements. The first two of these disintegrate, respectively, the red or the white corpuscles, the last-mentioned (or some of them) produce a precipitation in the serum. Something exactly similar occurs where an ordinary bacterial culture is inoculated. We obtain here in response to the inoculation of the bacteria, a development of bacteriotropic elements. These may, as we have seen, agglutinate, kill, dissolve, or otherwise disintegrate the bacteria. Further, in the case where the liquid medium in which the bacteria are suspended contains certain specific varieties of toxins, we may obtain, as in the case where diphtheria-toxin or tetanus-toxin is inoculated, toxitropic elements in the form of antitoxins which combine with and neutralise the poisonous properties of the toxins.

Inasmuch as, in the case of preventive inoculation against typhoid, we are concerned in particular to achieve the destruction of such typhoid bacilli as may effect an entrance

into the organism we shall do well here to consider certain questions which have relation generally to the disintegration and destruction of formed elements by antitropic substances, and in particular of bacteria by bacteriotropic elements, developed in the course of immunisation.

DISORGANISATION AND DESTRUCTION OF RED BLOOD-CORPUSCLES BY ERYTHROCYTOTROPIC SUBSTANCES AND OF BACTERIA BY BACTERIOTROPIC SUBSTANCES, AND SELECTION OF A VACCINE WHICH WILL INDUCE THE ELABORATION OF THE ANTITROPIC SUBSTANCES REQUIRED FOR THE DESTRUCTION OF THESE FORMED ELEMENTS.

It is impossible to conceive of formed elements furnishing, while they remain intact, a chemical stimulus, such as would induce an elaboration of antitropins complementary to the chemical constituents of those formed elements. The appropriate chemical stimulus will be furnished only when solution has taken place.

In point of fact it can be shown that, where foreign blood is introduced into the animal organism, the formed elements—in particular the red blood-corpuscles—are dissolved. Similarly it can be shown that, where typhoid bacilli are introduced into the blood or lymph, these also are dissolved.

The question of solution of the formed elements having in these particular instances been set at rest, we are immediately confronted with the question as to whether it is indispensable for the achievement of the destructive antitropic substances which we require, that the complex molecule which constitutes the chemical basis of the formed element should be introduced into the organism in an absolutely unaltered condition. It will be recognised that upon the answer which this question receives will depend the scientific justification for the employment as vaccines of bacterial cultures which have been chemically altered by heating, or by other agency.

It will be convenient to commence by ascertaining what modifications of the vaccine are permissible in the case of inoculation undertaken with the design of conferring upon an animal a power of hæmolysing a foreign blood.

We may, as Dr. Bulloch has recently shown, substitute for the intact or dissolved red corpuscles, which furnish the vaccine ordinarily employed, the stromata of the red corpuscles. We may go further still and may employ for our vaccine, in lieu of the fresh stromata, stromata which have been desiccated in an exsiccator.

These substitutions may, it appears, be made without any sacrifice of efficacy. In point of fact, it is not certain that the blood of an animal inoculated with a vaccine made from the desiccated stromata does not hæmolyse more effectively than the blood of an animal inoculated with intact red corpuscles or with the simple watery solution of blood.

Dr. Bulloch experimented further (*a*) with stromata which had been extracted with ether; (*b*) with the ether-extractive; and lastly (*c*) with the hæmoglobin apart from the stromata. The animals subjected to these inoculations did not in any case develop any hæmolytic power.

These experiments afford very interesting extension of data previously obtained in connection with bacterial vaccines.

The bactericidal, bacteriolytic and agglutinating powers which may be developed in animals by the inoculation of living typhoid cultures, and in man by the invasion of his organism by the typhoid bacillus, are obtained with equal facility by the inoculation of typhoid cultures which have been sterilised by heating.

Exactly the same thing applies in connection with the vibrio of cholera.

Mutatis mutandis, the same thing applies in case of the staphylococcus. The increased phagocytosis and the very favourable clinical results which I have obtained in acne, furunculosis, and sycosis by the inoculation of heated staphylococcus-cultures are evidence that a power of destroying the staphylococcus can be developed by the inoculation of heated cultures.

It is to be noted that, while it has been thus established that the power of disorganising bacteria can be quite well obtained by the inoculation of cultures which have been chemically altered by heating, it is manifest that a departure from the chemical constitution of the original culture is admissible only so far as it proves itself to be a modification which

leaves unaffected that chemical element in the vaccine which evokes the elaboration of destructive antitropic substances. It is of interest in this connection to note that the German Plague Commission[1] satisfied itself that the vaccinating power of a plague-culture is impaired or abolished when the customary addition of antiseptics is made to the unheated culture, instead of (as is ordinarily done) to the culture after it has been heated.

PHYSIOLOGY OF THE REACTION OF IMMUNISATION IN RELATION TO THE PROPER ADJUSTMENT AND PROPER INTERSPACING OF THE DOSES OF VACCINE.

The success of an inoculation-process does not in any case depend only upon the selection of the appropriate vaccine, upon the aseptic preparation of the vaccine, and upon the carrying-out of the inoculation in an aseptic manner. There is required further, upon the part of the operator, a certain comprehension of the physiology of immunisation, and, in particular, an apprehension of the general features of the reäction of immunisation.

LAW OF THE EBB, FLOW AND REFLOW, AND SUBSEQUENT MAINTAINED HIGH TIDE OF IMMUNITY.

There succeeds in every case upon the inoculation of a vaccine a *negative phase*, characterised by an impoverishment of the blood in antitropic substances. With this ebb, or negative phase, is associated a phase of increased susceptibility to bacterial infection, or, as the case may be, of increased sensibility to the toxic effect of the particular vaccine. This negative phase generally coincides with a period of greater or less constitutional distress.

The negative phase is succeeded by a *positive phase*, characterised by the flooding of the circulating blood with newly-formed antitropic substances. It may be presumed that this phase is associated with a maximum resistance to a bacterial invasion and a minimum sensibility to the poisonous action of the particular vaccine.

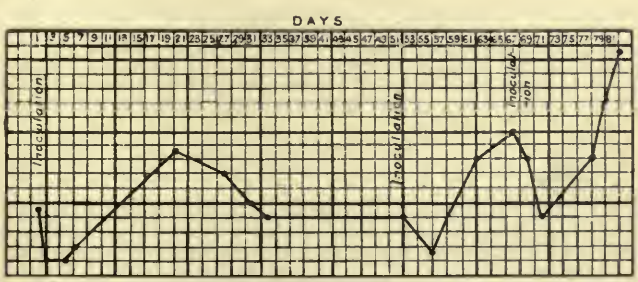
There supervenes upon the positive phase a reflow, or sinking away of the inflowing wave, leaving behind it a permanent, or relatively speaking permanent, high tide of immunity.

[1] *Report, German Plague Commission*, pp. 310-312.

During this period the blood shows, as compared with the period antecedent to inoculation, an increased content in antitropic elements. Moreover, the organism seems to possess during this period—and it would seem even afterwards—a greater power of response to a renewal of the vaccinating stimulus.

Basis of Experimental Fact upon which the Law of the Negative and Positive Phase rests.—Inasmuch as the law which has just been enunciated is a law of absolutely fundamental importance in connection with the physiology of immunisation it will be well to set out clearly the basis of experimental fact upon which it rests.

The law emerges for the first time in a paper of Ehrlich and Brieger,[1] in which there was set forth a curve of the reaction of immunisation. This curve, which is reproduced below, sets forth the variation in the amount of antitropic substances excreted in the milk from day to day during the course of immunisation. The particular experiment which furnished this tracing was an experiment in which tetanus-toxin was inoculated into a goat which had already by previous inoculations been rendered to some degree resistant to the effect of this poison.

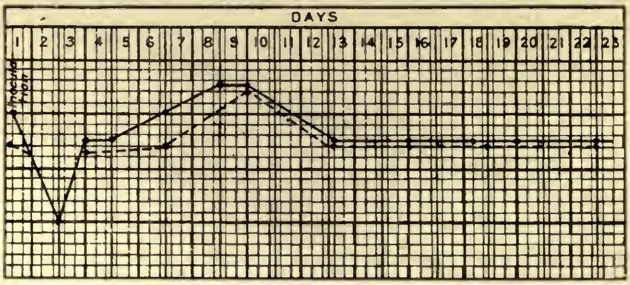


Curve obtained by Ehrlich and Brieger, showing the content of the milk in tetanus-antitoxin from day to day during the course of immunisation.

The same succession of negative and positive phase is disclosed in a curve of the reaction of immunisation, which is furnished by Salomonsen and Madsen.[2] In this particular

[1] *Ztschfts. f. Hygiene*, 1893.
[2] *Annales de l'Institut. Pasteur*, 1897.

case the curve sets forth the variation in the amount of diphtheria-antitoxin in the blood and milk of a mare during the course of immunisation.



Curve obtained by Salomonsen and Madsen, showing the content of the milk and blood in diphtherial antitoxin from day to day during the course of immunisation.

As yet the law had disclosed itself only as the law which governed the output of antitoxin from the antitoxin-forming centres.

I may perhaps claim to have been the first to bring out by my measurements [1] of the bactericidal power of the blood after typhoid inoculations, and of the phagocytic power [2] of the blood after staphylococcus-inoculations, the fact that the law of the negative and positive phase governs the production of bacteriotropic substances just in the same way as it governs the production of antitoxins (toxitropic substances).

Jorgensen and Madsen [3] adduced proof that the law of the negative phase applied also to the formation of the agglutinins of typhoid and cholera.

It was further shown by Morgenroth [4] in connection with the development of anti-rennet in the organism, and by Bulloch,[5] whose work was supplemented afterwards by the researches of Sachs,[6] that the production of these antitropic substances follows precisely similar lines.

[1] "On the Changes effected by Anti-typhoid Inoculation in the Bactericidal Power of the Blood." *Lancet*, Sept. 14, 1901.

[2] "On the Treatment of Furunculosis, Sycosis, and Acne by the Inoculation of a Staphylocccus Vaccine." *Lancet*, March 29, 1892.

[3] *Festskrift ved indvielsen af Statens Serum Institut., Kopenhagen*, 1902.

[4] *Centralblatt. f. Bakteriologie*, 1899.

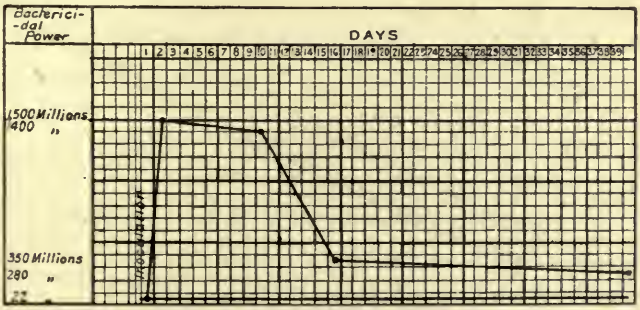
[5] *Trans. of Path. Soc. of London*, 1901.

[6] *Archiv. f. Anat. u. Phys.*, 1903.

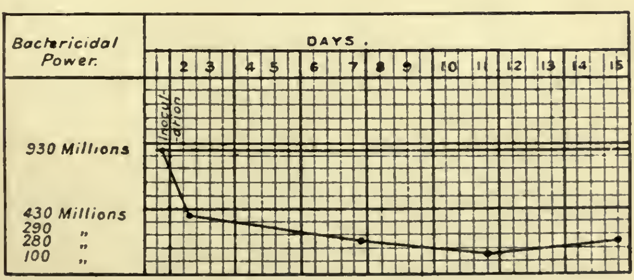
Thus by a series of successive extensions the law of the positive and negative phase has revealed itself to be the law which governs the production of antitropic substances generally. It is, in other words, the general law of immunisation.

DEPENDENCE OF THE NEGATIVE PHASE UPON THE DOSE OF THE VACCINE.

The intensity of the negative variation and the duration of this phase of diminished antitropic substances in the blood is, as I have been able to show in connection with the typhoid and staphylococcic inoculations, directly dependent upon the dose of vaccine administered. I have shown in the case of anti-



Elision of the negative phase. Curve obtained by the author, showing the content of the blood in bactericidal substances, in the case of a patient inoculated with a small dose of anti-typhoid vaccine.



Development of a prolonged negative phase. Curve obtained by author, showing content of the blood in bactericidal substances in the case of a patient inoculated with a large dose of anti-typhoid vaccine.

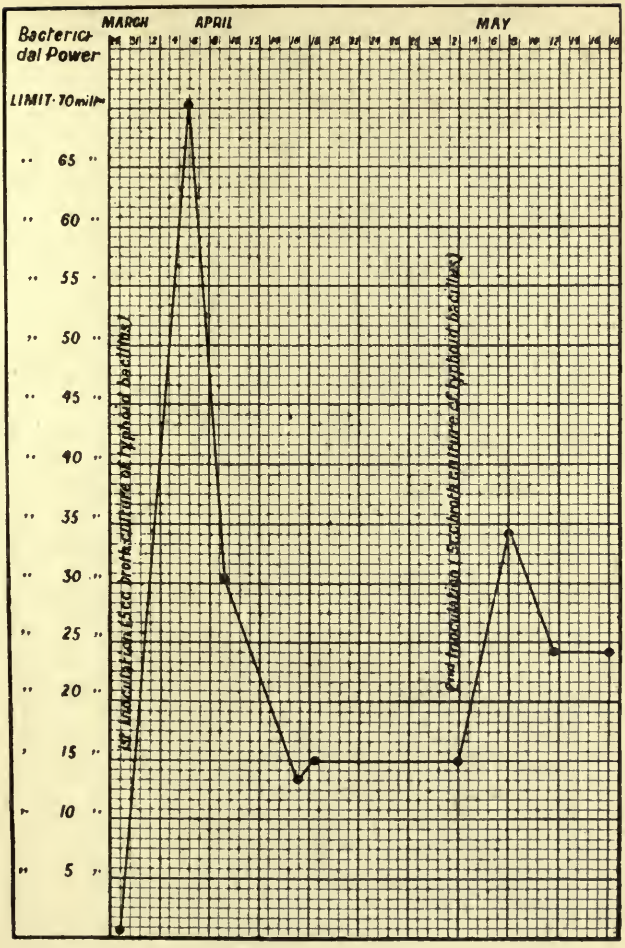
typhoid inoculation that where very small doses are administered—that is to say, doses insufficient to set up appreciable constitutional disturbance—the positive phase is already fully developed within twenty-four hours after the inoculation. A very fugitive negative phase has, it may be noted, quite recently come under the observation of Sachs in connection with the immunisation of animals against foreign blood. The curves which appear above illustrate respectively the elision of the negative phase and the prolongation of that phase. The differences here in question were dependent on the employment in the one case of a small, in the other of a large dose of vaccine.

CUMULATIVE EFFECTS OF TWO OR MORE INOCULATIONS.

Of great practical importance in connection with resort to repeated inoculation is a knowledge of the cumulative effect produced of two or more successful inoculations.

Cumulation may take place in two opposite directions. When in the case of two successive inoculations the second falls upon the positive phase of the former inoculation, or, as the case may be, on any period when the blood contains an increased quantity of antitropic substances, there is developed a cumulative high tide of immunity.

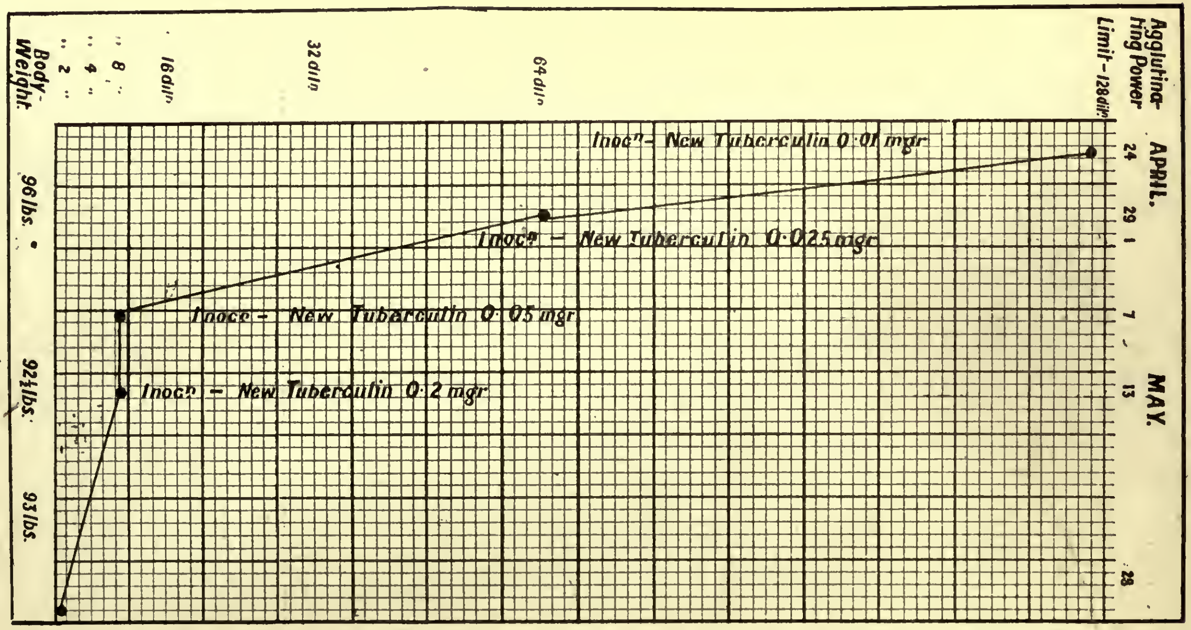
When, on the contrary, the second inoculation falls on the negative phase of the first, cumulation takes place in the direction of the negative phase. There is developed then a condition of cumulative susceptibility.



Cumulative effects of two inoculations properly interspaced. Curve obtained by the Author and Capt. W. Glen Liston, I.M.S., setting forth the content of the blood in bactericidal substances in the case of a rabbit inoculated with anti-typhoid vaccine.

In this and in the three preceding Curves the bactericidal power which is charted represents the bactericidal power of in each case 1 *c.c. of freshly drawn serum.*

The method employed was in each case the method described by the Author (Proc. Royal Society, Vol. 71*).*



Cumulative effects of a series of inoculations improperly interspaced. Curve obtained by the Author, setting forth the content of the blood in agglutinating substances in the case of a patient treated with tubercle vaccine (Koch's new tuberculin).

The method employed was the method described by the Author (Lancet, July 23, 1903).

CHAPTER II.

APPLICATION OF THE PRINCIPLES DEVELOPED ABOVE TO THE PROBLEM OF THE PRODUCTION OF IMMUNITY AGAINST TYPHOID FEVER.

It has appeared in Chapter I. that every condition of immunity is based upon the elaboration of antitropic elements in the organism. Here we pass on to the practical question as to how a condition of insusceptibility to typhoid infection may be achieved.

Taking it as a working assumption that we can compass our object only by inducing an elaboration of typhotropic substances in the organism, and by the inoculation of a vaccine prepared from a typhoid culture, our subject-matter distributes itself naturally under three headings. We have to ask ourselves :—

(*a*) What kind of services we desire the typhotropic substances, which are to be called into being, to undertake in the organism ;

(*b*) What form of culture, or what culture-derivative—what vaccine, in short—will induce the organism to furnish the antitropins which will be capable of undertaking the services required ;

(*c*) What procedures we ought to adopt in connection with the dosage, standardisation, and administration of the typhoid vaccine.

Before embarking upon the consideration of the first or these questions it will be well to realise the following facts with regard to the different ways in which antitropins may render protective service to the organism :—

(1) An antitropin may combine with the chemical element to which it stands in antitropic relation—we may hereafter speak of every such element as a *conjugin*—in such a manner as to throw it out of solution. An antitropin which undertakes this office is technically denoted a—*precipitin*.

(2) In the case where the conjugin is a poisonous element, the antitropin may combine with it in such a manner as to deprive it of its toxicity. An antitropin which undertakes

this office may, without reference to the derivation of its poisonous conjugin, be denoted an—*antitoxin.*

(3) In the case where the conjugin is a constituent of a formed element, an antitropin may combine with it as to cause the formed element to agglutinate when immersed in a sufficiently strong salt-solution.[1]

(4) An antitropin may, as I have recently shown in conjunction with Captain Stewart Douglas, I.M.S.,[2] modify the particular formed element to which it stands in antitropic relation in such a manner as to render it attractive pabulum to the white blood-corpuscles. An antitropin which performs this office may, as we have suggested, be denoted an—*opsōnin.*

(5) In the case where the conjugin forms one of the constituent bonds in the "vital ring" of the protoplasm, the combination of the antitropin with the conjugin may involve the shattering of the whole chemical structure of the protoplasm. An antitropin which combines with its conjugin in such a manner as to bring about this chemical disintegration may, in the case where bacteria are the formed elements under consideration, be spoken of as a *bactericidal substance.*

(6) An antitropin which disintegrates a complex molecule in such a manner as to resolve it into soluble elements, is technically denoted a *lysin.* When it brings into solution the hæmoglobin of the red blood-corpuscles, it is spoken of as a *hæmolysin.* When it brings into solution the protoplasm of bacteria, it is spoken of as a *bacteriolysin.*

(7) In the case where the conjugin forms a constituent bond in the "vital ring" of the protoplasm, and where it further constitutes, when set free, a poisonous element, the antitropin, while it shatters the structure of the molecule by entering into chemical combination with its conjugin, may at the same time quench the toxic properties of that conjugin. *An antitropin which fulfils this double office is at one and the same time a bactericidal substance and an antitoxin.*

NATURE OF THE SERVICES WHICH WE DESIRE THE TYPHOID ANTITROPINS TO UNDERTAKE IN THE ORGANISM.

What we desire from anti-typhoid inoculation is that it shall, if possible, ward off the typhoid attack; failing this, that it

[1] Attention may be recalled in this connection to the fact that agglutinating reactions manifest themselves only in the presence of salts.

[2] *Proc Roy. Soc.*, Oct. 1903, and Feb. 1904.

shall at least mitigate that attack. The successful achievement of the former object involves the devitalization of the typhoid bacillus immediately upon its entry into the system of the patient. The successful achievement of the latter involves the devitalisation of the typhoid bacillus, as soon as may be, after it has effected a lodgment in the system. For the attainment of either the former or the latter purpose, the system must be furnished with typho-tropic elements.

While the achievement of increased bactericidal power must in all cases be the primary object of concern, it is legitimate, as a secondary and subsidiary object, to aim also at the neutralisation of the toxic elements of the bacterial protoplasm, which are set free in the organism in the case where the typhoid attack is not completely warded off. This contingency will be provided for, if we can furnish the organism with antitropins which will perform, in addition to the office of devitalising the typhoid bacillus, also that of quenching the poisonous properties of the elements of the bacterial protoplasm, which pass into solution where the typhoid bacillus is broken up in the organism.

QUESTION AS TO WHAT FORM OF CULTURE, OR WHAT BACTERIAL DERIVATIVE, WILL INDUCE THE ORGANISM TO FURNISH THE TYPHOTROPIC SUBSTANCES WHICH ARE REQUIRED FOR THE PREVENTION AND MITIGATION OF TYPHOID FEVER.

In order to induce the organism to furnish the bacteriotropic substances which it will require when it is confronted by the typhoid bacillus, we must, as consideration will show, introduce into the body constituents of the protoplasm of the typhoid bacillus, as distinguished from the metabolic products which may have been elaborated by the micro-organism in the course of its cultivation.

Having recognised that the vaccine employed for anti-typhoid inoculation must contain constituents of the bacterial protoplasm, we have still to decide in what particular form these shall be administered.

In making our election between the different varieties of vaccines which might be brought into application we may take as our guide either tradition and *a priori* considerations, or,

emancipating ourselves from these, we may take as our guide the data furnished by a qualitative and quantitative determination of the antitropins which are elaborated in response to each particular variety of vaccine.

If conformity to the practice adopted in the earliest preventive inoculations were exacted, we should have to employ, as in the case of the Pasteurian inoculations against anthrax, and in the case of the anti-cholera inoculations of Haffkine—which were modelled upon the Pasteurian pattern—cultures of living attenuated microörganisms. It is unnecessary to point out that such a course would in the case of the typhoid bacilli which we are here considering entail grave risks :—the risk of disseminating the germ of the disease, and that of communicating the disease in a serious form in any case where by mischance the inoculated patient happened to be characterised by an abnormal susceptibility to typhoid infection.

If, while discounting the Pasteurian principle of the necessity of employing living micro-organisms, we were still to cling to the view that every method which involves a chemical alteration of the bacterial protoplasm is inadmissible, it would be necessary to resort to devices for devitalising our bacteria without exposing them to the action of heat or antiseptics.

Such a restriction would involve us in the grave inconveniences and risks which are associated with the proposed method of Macfadyen.[1] It would, on the one hand, necessitate resort to the complicated apparatus which is required for the trituration of bacteria ; and, on the other hand, it would deprive us of the security against contamination and against the risk of communicating the typhoid infection, which is obtained by the employment of cultures which have been devitalised by heat.[2]

If, lastly, we are prepared to accept evidence of the production of the desired antitropic substances as proof of the efficacy of the vaccine, we shall find ourselves, as the reader

[1] *Proc. Roy. Soc.*, 1903.

[2] It may be pointed out that the method of preparing typhoid vaccine which has been suggested by Macfadyen is essentially the same as the method previously employed by Koch in the preparation of his tubercle vaccine (new tuberculin). What has been said above with regard to the inconveniences and risks of Macfayden's method applies therefore, *mutatis mutandis*, also to Koch's method of preparing tubercle vaccine.

will realise at the end of the next section, and again when we come to deal with the statistics of anti-typhoid inoculation, free to employ for our inoculations cultures which have been sterilised by exposure to a temperature of 60° C.

It will be unnecessary to point out to anyone who has experience of such matters the manifold practical advantages which result from the employment of such sterilised cultures.

QUESTION AS TO WHETHER THE INOCULATION OF A TYPHOID CULTURE WHICH HAS BEEN STERILISED BY EXPOSURE TO TEMPERATURES OF 60° C. IS CAPABLE OF INDUCING IN THE ORGANISM THE ELABORATION OF THE TYPHOTROPIC SUBSTANCES WHICH ARE REQUIRED.

I may appropriately open the consideration of this question by pointing out that the suggestion that preventive inoculations should be undertaken against typhoid fever upon the Pasteurian system—a suggestion which was originally made to me by Mr. Haffkine—was, considering the risk which seemed to me to be involved in such a process, destined, so far as I was concerned, to remain indefinitely inoperative. The whole aspect of this suggestion was immediately changed as soon as I learned in the course of conversation with Professor R. Pfeiffer that he had in man obtained the specific agglutination-reaction to typhoid by the subcutaneous inoculation of a heated typhoid culture. This observation, since it pointed to the continued presence of effective vaccinating elements in the heated culture, immediately supplied the basis for the system of anti-typhoid inoculation which I have employed.[1]

In the course of the researches which have been carried on by me, so far as time and opportunity have allowed, during the last six and a half years, further evidence has accumulated in my hands with regard to the integrity of the vaccinating elements in typhoid cultures which have been subjected to the action of heat. The facts may be grouped under two headings :

[1] It may be observed that Professor Pfeiffer also recognised that his observation with regard to the production of agglutinins by inoculation of sterilised cultures had opened the way to anti-typhoid inoculation. The results of two experimental anti-typhoid inoculations were published by him in conjunction with Kolle (*Deutsche Medic. Wochenschft.*, Nov. 12, 1896) shortly after the publication of my first two anti-typhoid inoculations (*Lancet*, September 19, 1896).

—(*a*) facts showing that the typhoid culture is unaltered so far as its immunising properties are concerned by exposure to the temperature which is required for the devitalisation of the bacteria; and (*b*) facts showing that the chemical relations which obtain between the protective substances of the blood and the unheated typhoid bacillus, obtain also between these protective substances and the typhoid bacillus after it has been devitalised by exposure to a temperature of 60° C.

OBSERVATIONS WHICH SHOW THAT THE TYPHOID CULTURE PRESERVES ITS VACCINATING EFFICACY AFTER EXPOSURE TO TEMPERATURES OF 60°–65° C.

The proposition that the typhoid culture preserves its vaccinating efficacy after exposure to a temperature of 60° C. is established—

(*a*) by the fact that the bactericidal power of the blood is increased—sometimes as much as one-thousandfold—as the result of a single inoculation of a suitable quantum of a sterilised typhoid culture;[1]

(*b*) by the fact that an increased bacteriolytic power if developed in the blood of patients who have been inoculated with a suitable quantum of such sterilised typhoid cultures;[2]

(*c*) by the fact that a patient who has recovered from a first inoculation of a sterilised typhoid culture does not upon second inoculation suffer from the very severe constitutional intoxication which would supervene in the case of an uninoculated person inoculated with this dose of typhoid vaccine.

(*d*) by the fact that an increased opsonic power is developed in the blood of patients who have been inoculated with a suitable quantum of sterilised typhoid culture.

We have in (*a*) and (*b*) evidence of the elaboration of antitropins which exert a destructive effect on the typhoid

[1] Author's paper, *Lancet*, September 14, 1901.

[2] This may be demonstrated by making a series of progressive dilutions of the serum of a normal and of a vaccinated person, and mixing in each case the successive dilutions of serum with equivalent volumes of culture in a capillary tube. On examining microscopically films made with the contents of the capillary tube after the serum has been allowed to act upon the bacteria for half an hour at blood-heat, it will be found that the bacteriolytic effect has manifested itself in a much higher dilution in the case of the serum obtained from the inoculated patient, than in the case of the serum obtained from a normal person who acts as control.

bacillus; in (*c*) evidence of the elaboration of antitropins which discharge the office of antitoxins, and in (*d*) evidence of the elaboration of opsonic antitropins.

Summarising the above, we see that inoculation of cultures of typhoid bacilli which have been sterilised by exposure to a temperature of 60° C. induces in the organism an elaboration of—

(*a*) Agglutinating antitropins
(*b*) Bactericidal antitropins;
(*c*) Bacteriolytic antitropins;
(*d*) Antitoxic antitropins;
(*e*) Opsonic antitropins.

OBSERVATIONS WHICH SHOW THAT THE CHEMICAL RELATIONS WHICH OBTAIN BETWEEN THE TYPHOTROPIC SUBSTANCES OF THE BLOOD AND UNHEATED TYPHOID CULTURES OBTAIN ALSO BETWEEN THESE SUBSTANCES AND TYPHOID CULTURES WHICH HAVE BEEN EXPOSED TO A TEMPERATURE OF 60°–65° C.

The evidence under this heading is furnished by the fact that the agglutinating and bacteriolytic effects which are exerted upon living typhoid cultures by the typhotropic substances of the blood, are exerted also, but in a somewhat diminished degree, upon typhoid cultures which have been sterilised by exposure to a temperature of 60° C.

In sharp contrast with what occurs when the serum is brought into contact with cultures which have been sterilised at 60° C. is the entire absence of bacteriolysis in the case of cultures which have been heated to 72° C. before exposure to the action of the serum.

CONCLUSIONS WHICH CAN BE DRAWN FROM THE EVIDENCE FURNISHED UNDER THE TWO PRECEDING HEADINGS.

The concordant observations which have been set forth in the two preceding sections establish in a very clear manner that the vaccinating elements in the typhoid culture are maintained practically intact after exposure to a temperature of 60° C.

It is interesting to bring into relation with the experimental data with regard to the typhoid bacillus which have been

set forth above, certain experimental data relative to the red blood-corpuscles which have just been placed at my disposal by my friend Dr. Bulloch. In the course of experimentation upon the effect exerted by temperature upon the elements in the red blood-corpuscles which induce an elaboration of hæmolytic antitropins in the animal organism, it has been observed by Dr. Bulloch that the efficacy of these vaccinating elements is preserved, though it is somewhat diminished, by exposure to a temperature of 60° C. Exposure to a coagulating temperature of 72° C.—and it will be noted that this is the same as that referred to in the last section in connection with experiments upon the typhoid bacillus—completely abolishes the immunising properties of the red blood-corpuscle.

DIFFICULTIES WHICH STAND IN THE WAY OF THE RESOLUTION OF THE QUESTION AS TO WHAT FORM OF ANTI-TYPHOID VACCINE IS ABSOLUTELY THE BEST.

It will round off what has been said above in connection with the choice of a typhoid vaccine if I point out here that the problem as to what is the absolutely best anti-typhoid vaccine is a question to which the answer will be obtained only after very long and laborious experimentation. The sources of fallacy which are incident to an experimental investigation of the question may be brought before the reader's mind by the following analogy. While the character of the harvest constitutes conclusive proof that the proper variety of seed has been chosen, the fact that in a particular case the harvest has been plentiful, and has been specially rich in certain elements, does not constitute demonstration of particular virtues in the seed. The special character of the soil, the methods followed in the sowing, are capable of exerting a very sensible effect. In the same manner, the harvest of antitropins which is reaped in any particular instance will be influenced by the individual idiosyncrasy of the patient, and by the system of dosage which has been adopted. It must therefore be reserved for the future to determine whether the simple sterilised typhoid culture, such as I employ, constitutes the ideally-best anti-typhoid vaccine. For the present we must content ourselves with the fact that sterilised typhoid cultures have been shown to constitute a vaccine which will

induce elaboration of the special varieties of anti-tropins that are required for the protection of the organism against typhoid. It will presently emerge that the practical efficacy of the vaccine is borne out in a very distinct manner by the statistical records that set forth the results obtained by the actual practice of typhoid inoculation.

BRIEF ACCOUNT OF THE METHOD OF MAKING THE REQUIRED MASS-CULTURES OF THE BACILLUS TYPHOSUS AND PROCEDURES (A) FOR TESTING THE PURITY OF THESE CULTURES; (B) FOR STERILISING; AND (C) FOR DECANTING THEM WITHOUT RISK OF CONTAMINATION.

The detail of the technique which has been employed for obtaining a pure and perfectly sterile typhoid vaccine is reserved for the Appendix. It will suffice here to note that the description of the procedures which was given[1] by me in conjunction with my sometime colleague, Major W. B. Leishman, R.A.M.C., and which will be found reproduced in Appendix I., have stood the severe test which was imposed upon them by the demand for 400,000 doses of anti-typhoid vaccine which arose in connection with the South African campaign.

Evidence of the adequacy of the measures taken to secure the sterility of the vaccine is afforded by the fact that when, after the war, the unused vaccine was returned to store at Netley, the untouched bottles, so far as they were examined, were in every case found to be sterile. Perhaps even more satisfactory evidence of the adequacy of the system of technique which was employed is afforded by the fact that no case of septic trouble complicating inoculation was reported in connection with the inoculations undertaken in the course of the war. These inoculations may perhaps have numbered somewhere about 100,000.

STANDARDISATION OF THE VACCINE.

It is necessary to distinguish between standardisation and dosage; and it will be convenient to deal first with the former. Standardisation has for its object the determination of the

[1] *Brit. Med. Journal*, Jan. 20, 1900.

strength of one vaccine in terms of another vaccine, the dose of which has been determined by experiments undertaken upon man.

The following are the systems of standardisation which have from time to time been employed :—

(*a*) In the earliest inoculations, conducted in conjunction with Lieut.-Col. D. Semple, R.A.M.C., the virulence of the culture was in the first instance elicited by determining the fatal dose of that culture inoculated living into guinea-pigs of a standard weight of 250 grammes. The amount of sterilised vaccine employed for the inoculation was then adjusted in such a manner as to stand in relation with the amount of agar-culture which constituted the fatal dose for 100 grammes of guinea-pig.

(*b*) In the case of most of the vaccine sent out in connection with the South African war, a double process of standardisation was adopted. The toxic effect of the completed vaccine [1] was elicited upon a series of guinea-pigs. As a further control the opacity of the vaccine was measured by means of a simple and ingenious piece of apparatus devised for this purpose by my colleague, Major Leishman, R.A.M.C. [2]

Neither of these last-mentioned tests was completely satisfactory. It was found that the resistance of the guinea-pig to the toxic effects of the vaccine was an extremely variable factor, also that the measurement of the opacity was occasionally vitiated by autolytic processes which took place in the course of the cultivation. Thus it became advisable—and this was carried out wherever practicable—to control the tests which have just been described by the determination of the toxic effect exerted by the vaccine upon man. In particular it was found desirable to do this in the case where a typhoid culture which had been cultivated for prolonged periods on artificial media was replaced by a typhoid culture recently isolated from the organism.

(*c*) For the methods of standardisation described above I have recently substituted a much more satisfactory method. I now elicit the number of bacilli contained in the culture by

[1] This now consisted of a sterilised and carbolised broth-culture, which had been cultivated for 10 to 21 days.

[2] Figured in a paper by the author and Major Leishman, *Brit. Med. Journ.*, January 30, 1900.

enumeration[1] under the microscope. With a view to forestalling autolysis I have recently employed in every case 24-hour cultures. Again, in order to avoid the source of disturbance which is associated with the change of culture and with a view to facilitating enumeration, I now employ exclusively a strain of the typhoid bacillus which yields within a 24-hour limit of growth, under favourable circumstances, cultivations containing from 1,000 to 2,000 or more millions of bacilli in the cubic centimetre.

DOSAGE OF ANTI-TYPHOID VACCINE.

It will be manifest that the question as to what quantum of vaccine constitutes an appropriate dose is a question which can be determined only by the method of trial and error, *i.e.*, by the method of giving a series of graduated doses to a series of different individuals and selecting for employment the quantum which is found to be most appropriate.

In connection with the determination of the most appropriate dose, we may guide ourselves by reference to the clinical symptoms developed and in particular to the height of the temperature reäction. Or alternatively we may guide ourselves by reference to quantitative estimations of the content of the blood in antitropic substances.

In taking the clinical symptoms as a guide we are liable in the present state of our knowledge to fall into grave error.

(*a*) We may, for instance, in the case where an inoculation has failed to induce a temperature-reaction, draw from this fact the inference that the inoculation has been entirely unprofitable. Such an inference may be altogether erroneous. An elaboration of antitropins may in my experience occur quite independently of any temperature-reaction or other constitutional disturbance.

(*b*) We may fall into the error of believing that the more severe the constitutional reäction and the higher the fever, the greater must be the quantum of antitropins developed. Such an inference would be altogether erroneous. In point of fact very severe constitutional reaction is often associated with a

[1] The method of enumeration here referred to is that published by me in the *Lancet* for July 5, 1902. It is described in Appendix I.

prolonged impoverishment of the blood in protective substances. I have had experience of this in my own person in connection with the inoculation of a large dose of anti-typhoid vaccine. The observation made by Dr. Tooth in South Africa to the effect that a severe constitutional reaction had occurred in many of the inoculated who afterwards contracted typhoid fever, points in the same direction.

(*c*) When we have realised that the inference referred to under (*a*) is unwarranted, we may fall into the contrary error of assuming that a condition of immunity is being achieved in cases where the absence of constitutional reaction is in reality imputable to the inoculation of a defective vaccine or a deficient quantity of vaccine.

(*d*) When we have realised that the inference referred to under (*b*) is unwarranted, we may fall into the contrary error of inferring from the absence of fever that the quantum of vaccine which is being inoculated in a series of successive doses is not excessive. In the case set forth (Chart, page 14) there was nothing in the temperature to suggest that a cumulative negative phase was being induced.

We cannot safeguard ourselves against error in the dosage of vaccine except by making, after inoculation, a systematic series of blood-examinations upon the patients. In connection with experiments undertaken for the purpose of fixing the optimum dose—or, as the case may be, doses—of a vaccine, difficulties will present themselves which are in all respects similar to those which were under discussion on page 22 in connection with the question of the selection of the absolutely best vaccine.

These difficulties might perhaps be overcome in the case of a regiment or similar aggregate of men, by dividing it up into a number of batches, by inoculating each batch with a different quantum of the vaccine, and then constructing for each batch a curve representing the average course of the reäction of immunity in the batch. Such curves could be obtained by determining upon the pooled[1] blood of each separate group, the bactericidal, bacteriolytic, and agglutinating power which had been developed in the course of the reaction of

[1] Such pooled blood would be readily obtained by withdrawing, say, 50 cmm. of blood from each individual, and mixing these measured quanta.

immunisation. Having selected, after the consideration of the curves of immunisation thus obtained, the optimum dose—or, as the case might be, doses—of vaccine, it would be possible, given a perfect method of standardisation, to arrive without any further experimentation at the dose of any other typhoid vaccine which came to hand. Pending opportunity for carrying out such an experiment as that above described, I now employ in the case of the first inoculation a quantum of vaccine containing 750 to 1,000 millions of typhoid bacilli, and for the second inoculation a quantum of vaccine containing 1,500 to 2,000 millions of the same bacilli. I find that the inoculation of these quanta induces an ample elaboration of antitropic substances without producing any severe constitutional reaction.

QUESTION OF THE POSSIBILITY OF INTRODUCING THE VACCINE IN AN EFFECTIVE MANNER INTO THE ORGANISM BY THE CHANNEL OF THE ALIMENTARY CANAL.

In view of the considerable local pain which is induced by anti-typhoid inoculation, it is natural to enquire whether there may not be some method other than that of subcutaneous inoculation by which the vaccine may be introduced into the body. I was induced by a communication received from Captain C. E. Pollock, R.A.M.C., to take up again, in conjunction with Captain W. Glen Liston, I.M.S., in the summer of 1902, an investigation on this point which I had begun at an earlier date. This time my experiments were destined to be closured by the transference of the Army Medical School to London.

The gleanings from these experiments, which are set out in the curves on pp. 29 and 30, have, if I mistake not, at least a certain suggestive value.

Experiment 1.—The experiment here in question was undertaken upon Mr. F., a Surgeon on Probation in attendance on the Army Medical School, Captain W. Glen Liston, and myself. Mr. F. was a normal uninoculated man; Captain Liston had undergone anti-typhoid inoculation some two years before the date of the experiments; and I myself had on three occasions inoculated myself with anti-typhoid vaccine. The last of these

inoculations had been undertaken some 15 months before the date of the experiment.

The experiment was begun on June 1st by our drinking in each case 1 cc. of a typhoid vaccine, the dose of which had been fixed for the purposes of subcutaneous inoculation at 1 cc. On June 6th we drank a further 5 cc. of the vaccine, and on June 8th a further 15 cc.

Captain Liston and I did not suffer in any way from the inoculations beyond perhaps a little malaise. Mr. F., who was, it will be remembered, uninoculated, suffered from diarrhœa and considerable constitutional disturbance after imbibing the final dose of 15 cc.

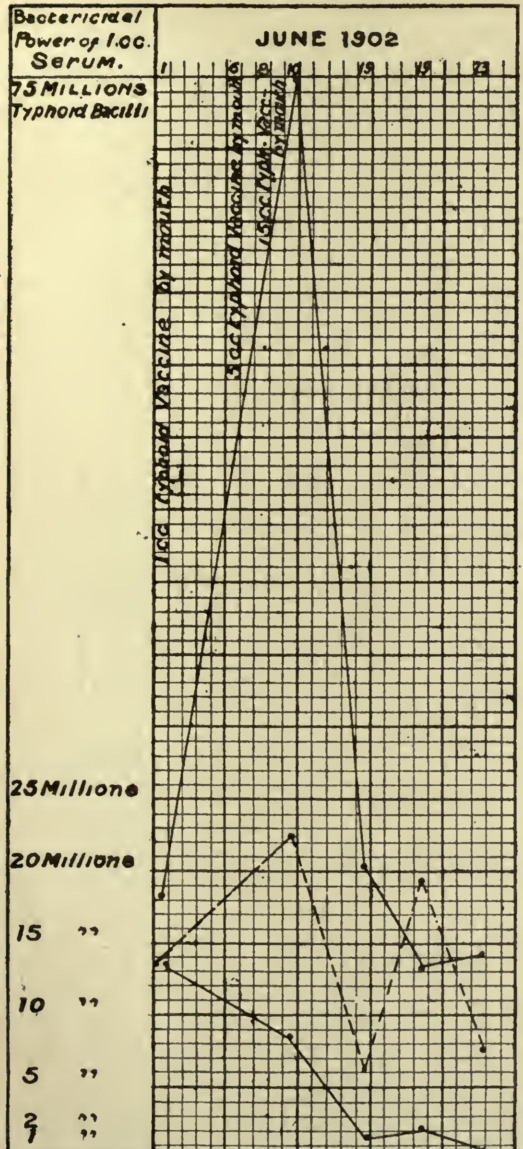
The observations made with regard to changes in the content of the blood in bactericidal substances are set forth in the curve on next page.

Commencing with the lowest of the three curves which represents the effect exerted upon Mr. F.'s blood, it will be seen that the ingestion of the anti-typhoid vaccine was followed by a very remarkable decline in the bactericidal power of the blood. We may most naturally interpret this as a negative phase similar to the negative phases which are produced by the subcutaneous inoculation of large doses of typhoid vaccine.

Passing to the middle curve, which represents the change that occurred in the bactericidal power of Captain Liston's blood, we see indications of the development of a moderate positive phase. Upon this supervenes a decline, followed again by a positive phase and a second decline. We may suppose that there was here some irregularity in the absorption of the vaccine from the alimentary canal.

In the case of the upper curve, which sets forth on a threefold-diminished scale the changes which occurred in the bactericidal power of my own blood, we have evidence of the production of a very striking positive phase, which was followed by a typical decline. It is interesting to note that this curve gives, so far as it goes, countenance to the suggestion made in Chapter I. (page 8, paragraph 4) that anti-typhoid inoculation may confer upon the organism a greater power of response to any renewal of the vaccinating stimulus.

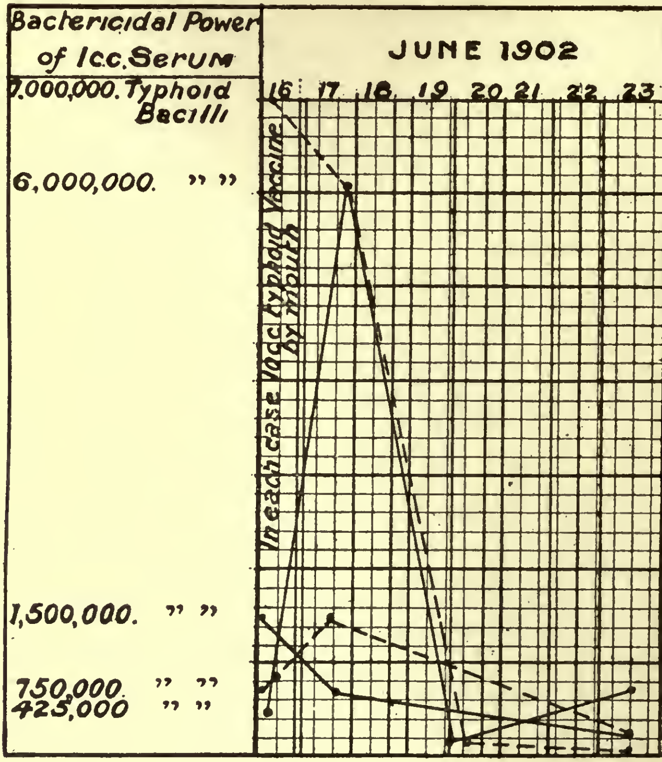
Experiment 2.—In the experiment here in question four Surgeons on Probation each drank 10 cc. of the same typhoid



Curve obtained by the author in conjunction with Capt. W. Glen Liston, I.M.S., setting forth the effect exerted on the bactericidal power of the blood by the ingestion of anti-typhoid vaccine by the mouth.

Upper Curve.—Effect exerted on author's blood—represented on a threefold diminished scale.
Middle Curve (broken line).—Effect exerted on Capt. Liston's blood.
Lower Curve.—Effect exerted on Mr. F.'s blood.

vaccine which was employed in Experiment 1. It will be seen, on reference to chart below, that in the case of two of these gentlemen a positive phase of very short duration was obtained. In the case of the other two gentlemen a negative phase only was achieved.

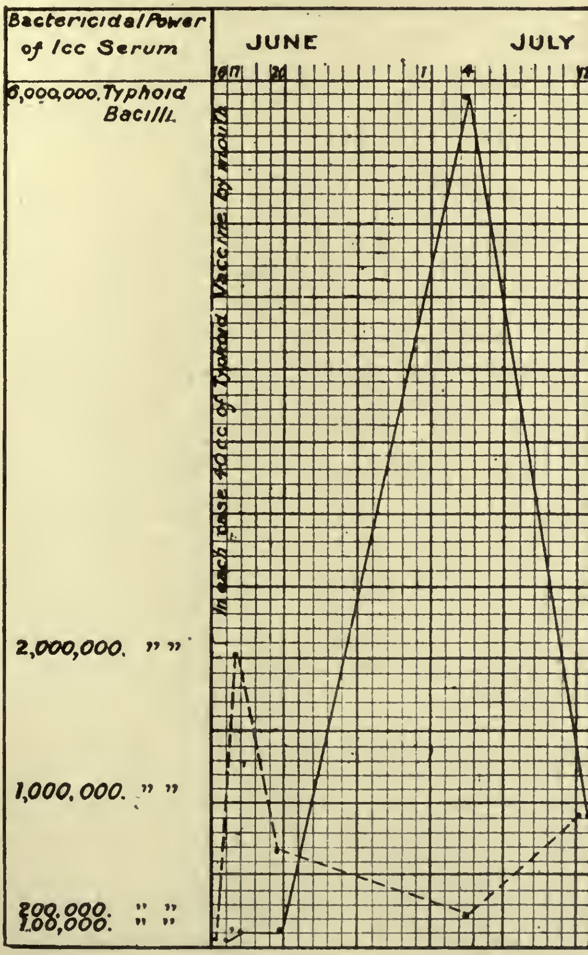


Curve obtained by the author in conjunction with Capt. W. Glen Liston, I.M.S., setting forth effects exerted on the blood of four uninoculated men by the ingestion of anti-typhoid vaccine by the mouth.

Experiment 3.—In the case of this experiment 40 cc. of the same typhoid vaccine was administered to each of two rabbits. The chart on the next page brings before the eye the result of this experiment.

We may surmise that the absence of negative phase may here stand in some relation to the resistance which the rabbit offers to typhoid infection.

Summary.—While the experiments on uninoculated persons set forth above do not seem to promise much in the way of immediate practical utility, it will be clear that the series of experiments suggests that the phenomenon of immunisation by acclimatisation, which is so strikingly exemplified by the statistical records of the British Army in India, may possibly be the result of an occasional ingestion of the typhoid bacillus into the alimentary canal.



Effect exerted on the blood of two rabbits by the introduction of anti-typhoid vaccine by the mouth.

QUESTION OF THE POSSIBILITY OF ACHIEVING PROTECTION APART FROM THE PRELIMINARY INDUCTION OF A NEGATIVE PHASE.

We have seen above in considering the general law which governs the elaboration of protective substances in the organism that there supervenes upon the inoculation of a vaccine into the organism in every case a negative phase.

It is not going too far to say that where the negative phase does not appear upon the curve of immunisation this is due either to too tardy examination of the blood, or to the fact that a dimunition of immunising elements in the blood cannot, by the nature of the case, be demonstrated where the blood contains at the date of inoculation only mere traces of the immunising elements in question.

We may take it as practically certain that under the conditions last referred to, it would be possible in every case—given that the methods for demonstrating its presence were available—to detect the vaccine in the circulating blood for a shorter or longer period after inoculation.

Such evidence has been obtained (*a*) in the case where the vaccine employed is characterised by well-marked toxic properties, (*b*) in the case where the vaccine can be detected by its specific power of reacting with a precipitating antitropin.

In the former case [1] toxic effects have been obtained in men and animals by the administration of serum drawn off from animals previously inoculated with various bacterial cultures or their derivatives—tuberculin, cultures of staphylococcus, streptococcus, micrococcus melitensis, &c., &c.

In the latter case [2] the vaccine has, after inoculation, been detected in the blood by the specific test. This has been done in particular by Von Dungern in connection with his injections of crabs' blood into rabbits.

It would seem clear, in the light of these further facts, that the negative phase in the curve of immunisation corresponds

[1] *Vide* Author's lecture (*Brit. Med. Journal*, May 9, 1903) on "Therapeutic Inoculations of Bacterial Vaccines;" in particular *vide*, the section dealing with the "Practical Importance of the Law of the Negative and Positive Phase in connection with the Therapeutic Inoculation of Sera derived from Animals vicariously inoculated with Bacterial Vaccines."

[2] *Vide* Von Dungern, *Die Antikörper*, pp. 81 and *seq.*, and his illustrative charts.

to a period of stimulation, just as the positive phase corresponds to the period of maximum response to that stimulation.

This once recognised, it will immediately be apparent that it will be as vain to expect to achieve a positive without a negative phase as to expect response without stimulation. It will be furthermore apparent that every suggestion which is directed to the *abolition* of the negative phase is a suggestion which involves as its corollary the assumption that a general physiological principle can be set aside.

These general principles having been elucidated, we may pass to consider the suggestions which Calmette and Besredka respectively have put forward in connection with the procedure of preventive immunisation.

The suggestion of Calmette has special reference to preventive inoculation against plague. It was to the effect that the serum of an animal which had been vicariously inoculated should be introduced into the organism of the patient at the same time as the bacterial vaccine. This suggestion here in question was based upon the three following premisses:—

(1.) Upon the premiss that protection is not acquired after the inoculation of bacterial vaccines until a period of fifteen days has expired.

(2.) Upon the premiss that the incorporation of serum from an animal which has been vicariously inoculated confers upon the patient a protection against bacterial invasion which lasts fifteen days.

(3.) Upon the premiss that it is possible to superimpose a condition of active immunity derived from the inoculation of a bacterial vaccine upon a condition of passive immunity derived from the incorporation of a serum in the manner in which a curve referring to an active immunisation can be mechanically superimposed upon a curve referring to a passive immunisation.

With regard to the first and second of these premisses, it will suffice to point out that abstract propositions such as these have no general applicability, and that they may be—and in point of fact are, as will be seen on referring to the curves of immunisation set forth in Chapter I.—quite out of conformity with the actual facts.

With regard to premiss No. 3, the following points may be noted:—

(*a.*) Inasmuch as the antitropic substances introduced in the serum of the vicariously inoculated animal would inevitably quench the chemical activity of the vaccine, we have no right to expect from the organism a response such as is obtained upon the introduction of a chemically active vaccine.

(*b.*) It has been shown by Jorgensen and Madsen[1] in connection with the production of typhoid agglutinins in the organism of animals that a combination of active with passive immunisation defeats its own object.

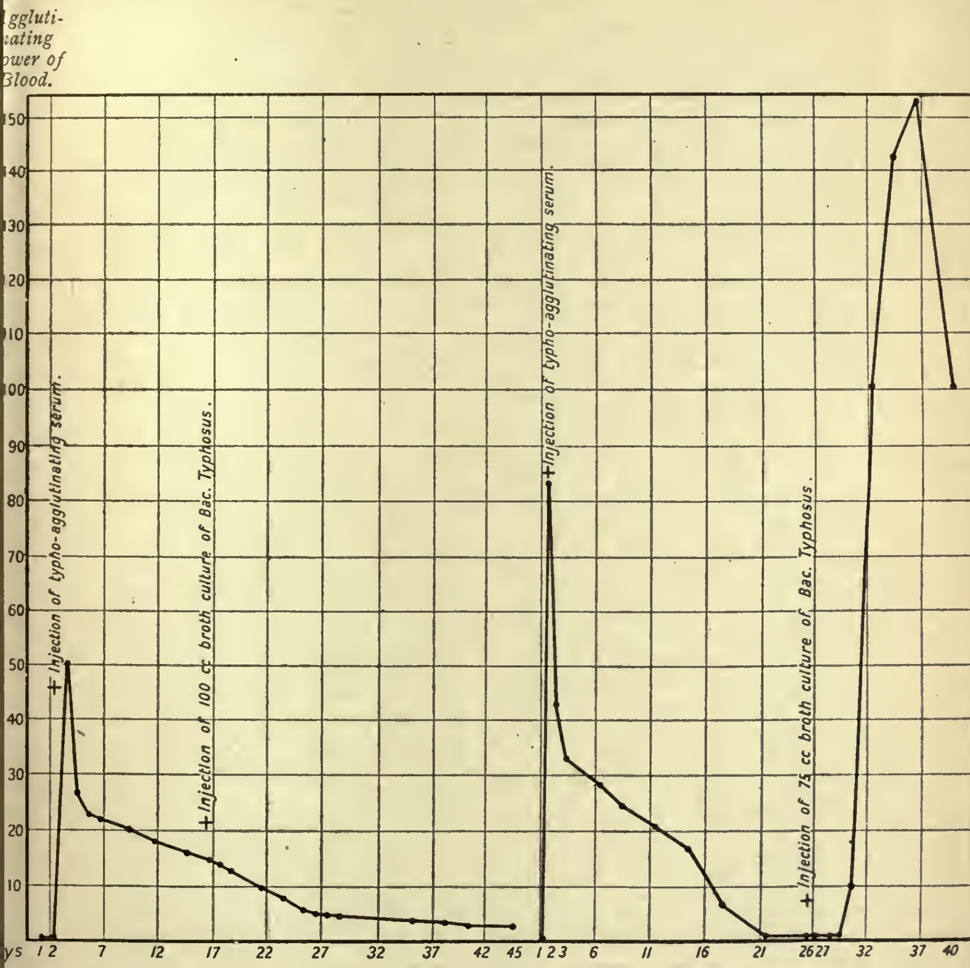
Two of the instructive curves which accompany Jorgensen and Madsen's paper are reproduced on pp. 35 and 36.

In a direct line of affiliation to the suggestion of Calmette is the suggestion of Besredka.[2] The suggestion of Besredka is that the bacterial vaccines which are employed in connection with anti-typhoid inoculation and in connection with other inoculations, should in each case be digested in vitro with sera obtained from vicariously inoculated animals, the surplus of serum being in each case removed from the vaccine by centrifugalisation and washing. It will be seen that this suggestion involves on the one hand a serious complication of the technique, and on the other hand a quenching of the chemical activity of the vaccine similar to that which is involved in the suggestion of Calmette.

Before dismissing the subject-matter of this negative phase it may be re-emphasised that, while the injection of a bacterial vaccine must in all cases be followed by a negative phase, that negative phase can at all times be regulated by apportioning the dose of the bacterial vaccine.

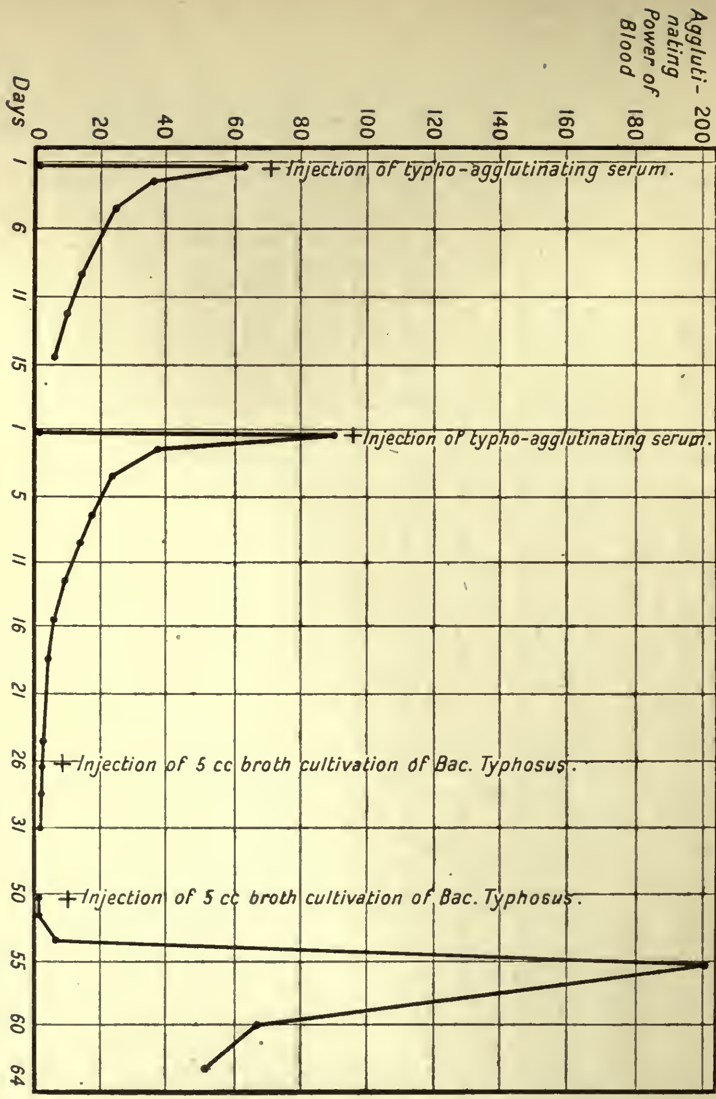
[1] *Loc. cit.*

[2] *Annales de l'Institut Pasteur*, 1902; *Comptes Rendus de l'Academie des Scienees*, June 20, 1902.



Curve obtained by Jorgensen and Madsen,[1] *showing, in the case of a goat,* (a) *the effect of superimposing an injection of a bacterial vaccine upon a passive (serum) immunisation, and* (b) *the effect of an injection of a bacterial vaccine undertaken after the expiration of the passive immunity.*

[1] *Festskrift ved Indvielsen af Statens Serum Institut, Kopenhagen,* 1902.



Curve obtained by Jorgensen and Madsen (loc. cit.) *showing, in the case of rabbits,* (a) *the march of events after the injection of serum from a vicariously inoculated animal ;* (b) *the effect of superimposing an injection of a bacterial vaccine upon a passive immunisation ;* (c) *the effect of an injection of a bacterial vaccine undertaken after the expiration of the passive immunity conferred by the serum-injection.*

Note.—For convenience of representation the agglutination curve is shown as reaching a climax in a sharp beak on the 55th day of the experiment. In reality a higher but undetermined level was reached between the 55th and 60th day.

CHAPTER III.

ON THE TECHNIQUE OF ANTI-TYPHOID INOCULATION, AND ON THE CLINICAL SYMPTOMS WHICH SUPERVENE UPON THE INJECTION OF THE VACCINE.

Having explained in Chapter I. the general principles of the physiology of immunisation so far as those principles have disclosed themselves to research, and in Chapter II. the application of these principles to the problem of immunisation against typhoid fever, we may here take up the consideration of the technique of anti-typhoid inoculation and of the clinical symptoms which follow inoculation.

ON THE TECHNIQUE OF ANTI-TYPHOID INOCULATION.

In connection with the technique of inoculation we have to consider the sterilisation of the syringe, the method of drawing off the vaccine from the containing vessel, and the selection and preparation of the site for inoculation. Printed instructions with regard to these points have in every case been issued along with the vaccine. These may be summarised as follows:—

(*a*) *Sterilisation of the Syringe.*—I have recommended that the syringe should in all cases be sterilised by filling it with oil heated to a temperature of about 140° C. I have pointed out that a piece of bread-crumb furnishes a convenient thermometer—the bubbling off of the steam indicating a temperature of over 100° C., and the browning of the bread-crumb after the steam has been driven off a temperature of 140° C. Where laboratory conveniences are not at hand, the oil with the bread-crumb thermometer may be conveniently heated in a tablespoon over an ordinary spirit-lamp. It is probably superfluous to point out that the filling of the syringe with oil at 140° C. secures instantaneous sterilisation.

(*b*) *Method of drawing off the Vaccine.*—In the case where the vaccine is contained in a capsule, the point of this latter

is, after sterilisation in the flame, to be broken off with a sterile instrument. The capsule having been inverted over the needle of the syringe, its contents may be withdrawn in an aseptic manner. In the case where the vaccine is sent out in a bottle covered in an air-tight manner by an india-rubber cap, the surface of the cap is conveniently sterilised by hot oil carried to it by the syringe. The vaccine may then be drawn off by puncturing through the rubber.

(c) *Choice and preparation of the site of Inoculation.*—It is advisable, with a view to avoiding the pain caused by the tension of the serous effusion which takes place at the point of inoculation, to insert the vaccine into a portion of the body where the skin is loose. The back of the shoulder and the flank are convenient situations. A fold of skin in one or other of these regions is picked up between finger and thumb, and the needle, after sterilisation of the skin with undiluted lysol, is carried down well into the loose subcutaneous tissue in the centre of this fold.

ON THE CLINICAL SYMPTOMS WHICH SUPERVENE UPON THE INJECTION OF THE VACCINE.

The character and the severity of the train of symptoms which supervene upon inoculation stand in relation with the dose of the vaccine administered. This is to be understood however with certain reserves. In the first place, it holds true only when we are comparing different doses of the vaccine made from one and the same strain of typhoid. In the second place, it probably no longer holds true after a certain limit of dose has been reached. I have, for instance, had it reported to me that the accidental inoculation (on a transport proceeding to South Africa) of tenfold the prescribed dose did not induce any greater effect than that which was induced by the inoculation of the already large dose actually prescribed.[1]

The clinical symptoms which supervene after anti-typhoid inoculation may be classed under the heading of (*a*) local

[1] This last point is theoretically of great interest, indicating, as it does, that the poisonous toxic elements in the vaccine, which evoke an elaboration of antitropic substances, combine with the cells of the organism in quite a different manner from the alkaloidal poisons. The effect of these last is in all cases proportional to the dose administered.

symptoms, *i.e.*, symptoms at the site of inoculation; and (*b*) constitutional symptoms.

Generally speaking, the severity of the local symptoms in cases of first inoculation is inversely as the severity of the constitutional symptoms. In cases where very severe constitutional symptoms have been produced, I have seen the local symptoms conspicuously absent.

Again, whereas in the case of the first inoculation constitutional symptoms have been well marked, second inoculations are frequently followed by nothing more than local symptoms.

Local Symptoms.—Where a suitable quantum of anti-typhoid vaccine, made from a suitable strain of typhoid bacillus, has been injected the local symptoms first make themselves felt after an interval of two or three hours. The effects then seen are the development of a red blush and more or less serous exudation at the seat of inoculation, followed by some lymphangitis along the lymphatics which lead, according as the vaccine has been inoculated above or below the middle line of the trunk, in the direction of the glands of the axilla or of the groin. In a case where a very toxic vaccine was employed, distinct local effects supervened in a quarter of an hour, the congestion around the site of inoculation afterwards assuming an almost erysipelatous intensity. Even such severe inflammation has never led on to suppuration.

It is a point of interest in connection with the pathology of the serous hæmorrhage which occurs at the site of injection that the coagulability of the blood is diminished in a very conspicuous manner as the result of anti-typhoid inoculation. The diminution is already very well marked within two or three hours after the incorporation of the vaccine.

With a view to counteracting the tendency to serous hæmorrhage at the seat of inoculation I have suggested the administration of 30 or 40 grains of calcium chloride. The restraining influence exerted by calcium chloride in these cases is analogous to that which I have shown it to exert upon the serous hæmorrhage of chilblains[1] and of certain forms of urticaria.[2]

[1] Author's paper, *Lancet*, January 30, 1897.

[2] Author's papers, *Brit. Journ. of Dermatology*, Vol. VIII., No. 89; *Lancet*, September 19, 1896; and *Transactions of Pathological Society*, Vol. 51, Part III., 1900.

Another point which has an importance in connection with the restraint of the effusion at the site of the inoculation is the circumstance that the ingestion of alcohol, or of any other substance which like alcohol[1] diminishes the coagulability of the blood, increases serous hæmorrhage and aggravates the local pain, or, as the case may be, brings it back after it has disappeared. Confirmation of my observations with regard to this point has reached me from many different quarters. I may point out that this increased serous exudation in the tissue which has been chemically injured by inoculation is in every way analogous to the exaggerated serous and actual hæmorrhage which is so familiar a feature in connection with mechanical injuries received in the condition of alcoholic intoxication.

Passing from the consideration of the causes which influence the serous hæmorrhage to the question of the treatment of the pain which is referable to it, attention may be drawn to the fact that it is greatly relieved by the application of warm stupes and by inunction with the following ointment :—

℞	Acidi Carbolici - - - -	gr. xx.
	Extract. Ergot. Liquid. - - - -	ʒ iss
	Zinci Oxidi - - - -	ʒ i
	Lanolin - - - - - - -	℥ i

Two further points may be noted in connection with the effect at the site of inoculation. The first of these is for two or three weeks after inoculation a hard nodule, about the size of a large pea, may be felt on rolling the subcutaneous tissue between the finger and thumb.

The second, and this is a theoretically interesting point, is that it has been reported to me both from India and from South Africa that the site of inoculation has in isolated instances become painful in the case of patients who have contracted typhoid within a few months subsequent to inoculation.

Constitutional Symptoms.—Constitutional symptoms generally supervene on inoculation within the space of two or three hours. Upon one occasion, when experimenting at

[1] Author's paper, *British Medical Journal*, July 14, 1894.

Netley on four professional colleagues, I have seen rigors supervene in each case within a quarter of an hour. The vaccine inoculated in these cases consisted of one quarter of a cubic centimeter of a sterilised broth-culture of a typhoid bacillus which had been recently isolated from a patient dead of the disease.

It is to be noted that the onset of the constitutional symptoms is hastened and the severity of these symptoms is increased where any muscular exercise is engaged in immediately after inoculation.

It has also appeared to me that inoculation undertaken upon a fasting patient is followed much more rapidly by constitutional symptoms and also by symptoms of a severer type. This last observation, be it noted, has a considerable theoretical interest. The reader who is familiar with Ehrlich's theory of immunity and with von Dungern's experiments [1] in connection with the immunisation of rabbits against the blood of a particular variety of crab will recognise that the occupation with food of the receptors which subserve the nutrition of the cell may quite well delay the incorporation of the toxic elements of the vaccine with the cell-protoplasm.

With the doses of vaccine which I now employ—doses which I have said are quite sufficient to elicit a satisfactory elaboration of typhotropic elements—the constitutional symptoms are limited to some headache, and to two or three hours of real malaise. As soon as these symptoms have passed off—generally five to six hours after the inoculation—the patient feels inclined for food and for sleep. The sleep which is obtained is of course somewhat broken. Next day his temperature comes down to normal, and he feels comparatively well except in the respect of pain at the seat of inoculation.

With the larger single dose which was employed during the South African campaign symptoms of collapse and rigors were not unfrequent. These symptoms, although to the experienced eye they in some cases appeared alarming, in no case led to any accident.

Recovery appears to have been slow in some cases where severe constitutional symptoms were produced. It may be

[1] V. Dungern: *Die Antikörper*, p. 103, Jena, 1903.

surmised that such delayed recovery was associated with a prolongation of the negative phase.

The practical conclusion which is to be drawn from the above is that, where circumstances allow of this being done, it is advisable to employ, instead of the one severe inoculation (which had under the circumstances to be employed in my inoculations in India and in the case of most of the inoculations undertaken upon soldiers proceeding to South Africa), two successive inoculations, adjusted so as to avoid the supervention of severe constitutional symptoms. Experience further shows that the patient may with advantage partake of some light nourishment immediately before or immediately after anti-typhoid inoculation ; and that he should avoid all physical exertion after inoculation. He may in fact with advantage betake himself to bed or in winter to an arm-chair by the fireside as soon as possible after the injection. A dose of calcium chloride may also be administered to him.

CHAPTER IV.

ON THE PROTECTIVE EFFECT OF ANTI-TYPHOID INOCULATION AS EXHIBITED IN THE STATISTICAL RECORDS.

Before addressing ourselves to the study of the statistical records of anti-typhoid inoculation we may with advantage consider certain fundamental points which concern the cogency of statistical evidence.

1. In every case where an enquiry is to be made as to whether a particular inoculation exerts a protective effect, it is essential that there should be included in the observation along with a group of inoculated men also a control group of uninoculated men. The control group, which ought to correspond with the inoculated group in all points save only in the circumstance of inoculation, is required for the purpose of furnishing information as to what, in the absence of inoculation, would have been the incidence and the case-mortality of the particular disease under consideration among those who are included in the inoculated group.

2. It is further essential that there should be placed upon record the exact numbers of both the inoculated and uninoculated groups, and the percentage incidence, and case-mortality of each group.

FALLACIES AND ERRORS WHICH ARE INCIDENT TO STATISTICAL RECORDS SUCH AS THOSE WHICH COME INTO CONSIDERATION IN CONNECTION WITH ANTI-TYPHOID INOCULATION.

To the reader who has not had to concern himself with furnishing or, as the case may be, with sifting statistical evidence, it will perhaps appear a very simple matter to comply with the elementary statistical canons formulated above. In point of fact experience teaches that the code can never be wholly conformed to. It follows—and this ought to be put in the forefront in considering any statistical evidence—that the exploitation of statistics without critical discussion furnishes proof either of the *naïveté* of the statistician, or of a desire to

turn to advantage that inborn distaste for intellectual effort, which is the most characteristic feature in the psychological organisation of man.

The more important of the complexities which encumber statistical evidence, such as we have here to deal with, are the following :—

1. *It is hardly ever practicable to obtain in an absolutely accurate manner the respective numbers of the inoculated and uninoculated.*

This seemingly paradoxical proposition is justified by the following considerations :—

(*a*) The number of men in inoculated and uninoculated groups is almost inevitably subject to variation during the course of the period covered by the observation. In the case, for instance, of a regiment in India or on service new drafts arrive to join the regiment, time-expired men and invalids return home, and occasional transfers are made. The statistical difficulty which thus arises can be surmounted only by recording for each inoculated and uninoculated man the number of days in which he has been under observation with the regiment.

It will be noted on consulting the critical commentary appended to the statistical table given below that this statistical method has superseded in the case of the Indian statistics for the year 1901 the uncritical method of enumeration employed in the two preceding years.[1]

(*b*) In the case where inoculations are undertaken in the course of an epidemic, we have to deal with a group of uninoculated decreasing *de die in diem*, and with a group of inoculated increasing in the same manner.

This statistical difficulty, and the difficulty arising from the associated spontaneous rise and fall of the epidemic, can be surmounted only by calculating the incidence upon the average strength of the two groups during the period of observation, and by calculating the risk of infection before and after the date upon which the average strength was reached. The somewhat intricate principles of this method of computation will be found fully discussed in the chapter of the Indian

[1] The introduction of this more critical method of enumeration is due to the inspiration of Major T. MacCullagh, R.A.M.C., the present statistical officer at the head-quarters of the Army Medical Department.

Plague Commission (Chapter IV., para. 434) which deals with the statistical results of Haffkine's anti-plague inoculations. The method finds exemplification in a paper[1] in which I have set forth the results obtained by anti-typhoid inoculation in the case of an epidemic of typhoid fever which took place in the Richmond Asylum, Dublin.

(*c*) In the case where subsequent to inoculation, but within the limits of the incubation-period, cases of the disease occur a difficulty arises as to how these cases are to be brought upon the record.

It is traditionary with the medical statistician to exclude all such cases from consideration. I think this practice is to be deprecated—first, because it can never be certain in the case of an inoculated man that his susceptibility to the disease has not been temporarily increased by inoculation ; secondly, because it is important to have set forth the balance of advantage and disadvantage of inoculation undertaken in the actual presence of infection.

2. *It is a matter of great difficulty to secure the exact comparability of the inoculated and uninoculated groups.*

It was laid down above as one of the theoretical requisites in connection with statistics that the control group should correspond with the inoculated group in all points save only with respect to inoculation. We are compelled in actual practice to abate something from this theoretical requisite. But it is in all cases essential that the control group shall consist of persons who are similar to the inoculated with respect to age, and of persons who live under similar conditions, and who are exposed for the same period to the similar risks of infection.

When these essential points have been neglected the statistical conclusions arrived at are entirely without coercive force.

Illustration of the vitiation of statistical records by omission to consider the question of the comparability of the inoculated and uninoculated groups is furnished by the statistical record No. 22 in the synoptical table, pp. 54 and 55.

The next-following statistical record furnishes illustration of the fact that a statistical conclusion arrived at by a comparison of the inoculated with a control group selected at random may

[1] *Brit. Med. Journ.*, October 26, 1901.

be the exact reverse of the conclusion which emerges from a comparison with a properly selected control group.

3. *It has not always been possible to secure a correct assignation of the sick to the inoculated and uninoculated groups respectively.*

The determination of the point as to whether a particular patient belongs to the group of the inoculated or uninoculated would also at first sight appear to be an absolutely simple operation in which it would be impossible to go wrong. This may be taken to be so in the case of soldiers where the fact of inoculation has been entered upon the Medical History Sheets, and where, as in time of peace, the Medical History Sheets are available. Quite other were the conditions in the case of the men entered as inoculated or uninoculated respectively on the records of many of the Military Hospitals in South Africa. The classification of the patient as inoculated or uninoculated depended here upon his own statement. A serious source of error was thus introduced. In certain cases where the soldier desired to avoid inoculation, and suspected that this would follow if he returned himself as uninoculated, a strong motive existed for making a false return. In other cases, again, confusion arose in the man's mind between anti-typhoid inoculation and anti-smallpox vaccination, the confusion being due to the fact that both these procedures were undertaken on the transports on passage to South Africa.

In addition to these sources of misclassification there were many others which are best passed over in silence.

4. *In a considerable percentage of cases it is notoriously a matter of difficulty to arrive at a certainty in the differential diagnosis of typhoid fever.*

The difficulty of the differential diagnosis of typhoid fever from other fevers is notorious. In the case of the army, and in particular in the case of the army on field service, there exists a very intelligible bias in favour of arriving at definite diagnoses; and the diagnosis which is most in favour in the case of fevers is the diagnosis of typhoid fever.

It follows that, even putting other difficulties aside, the difficulties associated with diagnosis would make absolute accuracy unattainable in statistics referring to the effect of anti-typhoid inoculation.

The effect of the inclusion of these doubtful cases, or, as we may perhaps conveniently call them, *pseudo-typhoid* cases, in the statistical records will, as consideration will show, operate to the prejudice of reputation of anti-typhoid inoculation. It will operate in this manner, inasmuch as the protection will be judged to have failed, not only when the patient contracts typhoid fever, but also when he contracts any kind of which is confused with typhoid fever.

5. *It is difficult to ensure accuracy in the recording of the facts.*

Up to the present we have had under consideration errors which partake of the nature of fallacies as distinguished from errors arising from sheer inaccuracy and inadvertence. These last, however, can never be completely avoided, and it behoves the statistician in all cases to make careful enquiry for them. On consulting the critical commentary appended to the statistical table below, it will be seen that more or less serious inaccuracies have been detected in connection with four or five of the statistical records.

QUESTION AS TO WHAT STATISTICAL EVIDENCE MAY BE ACCEPTED AS COERCIVE.

In view of the fact that flaws will reveal themselves, if only they are diligently and conscientiously sought for, in all statistical evidence, the question urgently presents itself as to when and under what circumstances the evidence of imperfect statistics as the protective effect of an inoculation may be accepted as coercive.

The reply that will be made to this question by persons gifted with very great critical acumen will be that it never becomes coercive. Persons who desire to wait till the evidence is obtained which satisfies the theoretical canons set forth at the outset of this statistical discussion are persons who desire to wait indefinitely. The plain, everyday man will find it possible to reconcile the demands of his statistical conscience with the demands of practical life. He will neglect the mint and anise and cummin of statistical criticism while holding fast to weightier principles of the statistical law.

The essential principles of this law may be compressed into the following propositions :—

1. *Where a flaw has been detected in a statistical record the effect of that flaw upon the cogency of the statistical conclusion is to be evaluated. The statistical conclusion in question is to be upheld where the error is shown to be one which is incapable of exerting an appreciable influence on the result.*

Example 1.—In the case of the statistics relating to the effect of anti-typhoid inoculation in the garrison of India for the year 1900 (Synoptical Table, serial number 6), there is a confessed error in the enumeration of the inoculated. That error may, it is estimated, amount to "some hundreds." The proper way to deal with this situation is, not to set aside the statistics, but to consider instead what would be the maximum effect which an error of this particular magnitude is capable of exerting upon the statistical conclusion. Interpreting the error of "some hundreds" in the enumeration of the inoculated in a liberal spirit as an error of 600 one way or the other, the error in the enumeration of the group would amount to a 10-per-cent. error. This particular statistical conclusion is thus seen to be subject to a 10-per-cent. error. In relation to the broad issues which are here under discussion such an error is insignificant. It is a matter of quite subordinate interest whether the diminution of mortality in the inoculated was the 4·4-fold diminution which is actually registered, or a 4·8-fold, or only a 4-fold diminution.

Example 2.—An even more striking illustration of the importance of working in accordance with this statistical principle is afforded by the statistics relating to the garrison of Egypt for 1900 (Synoptical Table, serial number 5). Here we have recorded an 18-fold diminution in the incidence of typhoid fever among the inoculated, numbering 700 odd, as compared with the uninoculated taken as some 2,600 odd. If we include in the number of the inoculated those inoculated in the previous year, who are specifically excluded from the statistical returns here in question, we arrive at an inoculated strength of over 1,000 and obtain a 25-fold diminished incidence. If, again, waiving the legitimacy of this course, we include among the uninoculated the full number of troops

who passed through Egypt in the course of the year in connection with military movements to South Africa, we bring up our uninoculated strength to some 5,000 and obtain only a 13·5-fold diminution. For settling the broad issue which we have in view in this paper it matters nothing whether we accept the highest or the lowest of the above figures as representing the diminution in typhoid incidence achieved in the inoculated.

2. *Where a flaw has been detected in a statistical record and where it has been shown that the error in question is an error of such magnitude as to be capable of reversing the statistical conclusion arrived at, the statistical record in question is to be rejected, except in the case where data are accessible which allow of the correction of the error.*

Examples.—Examples of statistical flaws which invalidate the conclusion arrived at are furnished by Dr. Melville's statistics and Dr. Crombie's first statistics (Synoptical Table, serial numbers 7 and 22 respectively). Dr. Crombie's second statistics, as embodied in the Synoptical Table below (serial number 23), furnish an example of a statistical record which may be accepted after it has been corrected in the manner provided for by its author.

3. *Where the figures are large, all chance errors which may have been committed in the enumeration of the inoculated and uninoculated or in the classification of the sick are spontaneously eliminated, and the statistical conclusions which emerge may so far be accepted with confidence.*

When the figures are small the statistical conclusion is uncertain even in the case where the facts have been accurately recorded.

Examples.—The figures (Synoptical Table, serial numbers 2, 6, and 25) for the inoculated and uninoculated in the garrison of India for each of the three successive years 1899, 1900, and 1901 are sufficiently large to eliminate the operation of chance.

The figures (Synoptical Table, serial number 13) for the case-mortality of the inoculated and uninoculated collected by Dr. Dodgson from various Military Hospitals in South Africa are sufficiently large to eliminate the operation of chance.

The figures for the case-mortality of the inoculated and

uninoculated obtained by Drs. Elliot and Washbourn (Synoptical Table, serial number 12) are too small to insure the elimination of the operation of chance.

4. *Where there is a source of error which runs through all the statistics and operates always in one and the same direction, that error is not eliminated by the cumulation of figures.*

Example.—The error which is introduced into the statistical records by the inclusion of *pseudo-typhoid* cases among the typhoid cases is not eliminated by the accumulation of figures.

It will be remembered that this is an error which operates to the prejudice of the reputation of anti-typhoid inoculation.

SYNOPTICAL TABLE OF ALL THE STATISTICAL RECORDS RELATING TO ANTI-TYPHOID INOCULATION.

The Synoptical Table below consists essentially of a reprint of the table which I embodied in my statistical paper published in the *Lancet* of Sept. 6th, 1902. I have brought it up to date by adding to it three further statistical records which have become available since the publication of my former paper. As explained in connection with my former publication, I have in my table in each case set out the statistical materials in the form in which those materials were furnished by their authors, except only in the respect that I have incorporated with them, whenever such were available, data with regard to the dates of the observations and certain other points of interest.

To the Synoptical Table I have appended a critical commentary. In this I have set forth, but somewhat less fully, the facts which were furnished in Table II. of my previous paper. I desire to emphasise that the data given in the Synoptical Table ought to be studied in the light of the critical commentary.

I.—STATISTICS OF ANTI-TYPHOID INOCULATION.

I.—STATISTICS OF ANTI-

To be studied in Connection with the

Serial Number.	Group which was under Observation.	Interval between Inoculation and Commencement of Period of Exposure.	Place where Group was exposed to Infection.	Period of Observation covered by the Statistics.	Authority for the Statistics.	Reference to published Literature or other Source of Information.	Number of Inoculated and uninoculated in the Group.	
							Inoculated.	Uninoculated.
1	Nurses and Attendants, Barming Asylum.	No Interval.	Barming Asylum, Maidstone.	Oct. to end of epidemic. 1897.	Dr. J. S. Tew. Mr. A. G. R. Foulerton. Dr. A. M. Jackson.	*Public Health*, Mar., 1898. *The Lancet*, June 2, 1900.	84	116
2	Inoculated Regiments and other Units of the British Army in India.	No interval.	Various Stations in India.	1899	Official returns.	*Army Medical Report* for 1899.	4,502	25,851
3	15th Hussars, wives of same, and officers.	Probably a few weeks.	Meerut, India.	Oct., 1899, to Oct., 1900.	Official returns.	*Brit. Med. Jour.* and *The Lancet*, Feb. 9, 1901; reported by Prof. A. E. Wright.	360	179
4	Garrison of Ladysmith.	Two months to 11 months.	Ladysmith, South Africa.	Nov. 2, 1899, to Feb. 28, 1900.	Official returns.	*Brit. Med. Jour.* and *The Lancet*, July 14, 1900; reported by Prof. Wright.	1,705	10,529
5	British Garrison of Egypt and Cyprus.	No interval.	Egypt and Cyprus.	1900	Report of Principal Medical Officer, Egypt.	*Brit. Med. Jour.* and *The Lancet*, May 4, 1901; reported by Prof. Wright.	720	2,669
6	British Garrison in India.	Varying from no interval to one year.	India.	1900	Official returns.	*Army Medical Report* for 1900.	5,999	54,554
7	Patients in Tintown Hospital, Ladysmith.	No data.	South Africa.	—	Dr. D. Melville.	*Brit. Med. Jour.*, Vol. I., 1901.	—	—
8	Patients in Stationary Hospital, Harrismith.	"	South Africa.	Sept., 1900. to Sept., 1901.	Maj. C. Birt, R.A.M.C.	*Brit. Med. Jour.*, Jan. 11, 1902.	—	—
9	Patients in Portland Hospital.	"	South Africa.	1900.	Dr. H. H. Tooth.	*Brit. Med. Jour.*, Mar. 16, 1901.	—	—
10	Patients in the Irish Hospital.	"	South Africa.	1900.	Dr. J. B. Coleman.	*Transactions of the Royal Academy of Medicine in Ireland*, Vol. XIX.	—	—
11	Patients in Scottish National Red Cross Hospital.	"	South Africa.	1900	Colonel Henry Cayley, I.M.S.	*Brit. Med. Jour.*, Jan. 12, 1901.	—	—

TYPHOID INOCULATION.

Critical Commentary appended.

Number of Cases of Typhoid Fever.		Percentage Incidence of the Disease.		Number of Deaths from Typhoid Fever.		Percentage Death-rate for Typhoid Fever.		Case Mortality—*i.e.*, Proportion of Deaths to Cases.		Supplementary Facts.
In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	
0	4	0	3·4	—	—	—	—	—	—	12 cases of typhoid fever had occurred in the staff, numbering about 200, before the inoculations were undertaken.
44	657	0·98	2·54	9	146	0·2	0·56	1 in 4·9	1 in 4·5	—
2	11	0·55	6·14	1	6	0·27	3·35	1 in 2	1 in 2·2	—
35	1,489	2·05	14·14	8	329	0·47	3·12	1 in 4·7	1 in 4·5	—
1	68	0·14	2·55	1	10	0·14	0·37	1 in 1	1 in 6·8	—
52	731	0·87	1·69	8	224	0·13	0·58	1 in 6·5	1 in 3·3	—
30	265	—	—	2	5	—	—	1 in 15	1 in 53	—
263	947	—	—	18	135	—	—	1 in 14·6	1 in 7	A comparison of 151 charts referring to inoculated patients with 317 charts referring to uninoculated patients showed that the height of the fever, the duration of the same, the percentage of relapses, and the number of daily evacuations were much less in the inoculated.
54	178	—	—	4	25	—	—	1 in 13·5	1 in 7	—
80	592	—	—	5	74	—	—	1 in 16	1 in 8	—
15	70	—	—	1	10	—	—	1 in 15	1 in 7	—

Serial Number.	Group which was under Observation.	Interval between Inoculation and Commencement of Period of Exposure.	Place where Group was exposed to Infection.	Period of Observation covered by the Statistics.	Authority for the Statistics.	Reference to published Literature or other Source of Information.	Number of inoculated and uninoculated in the Group: Inoculated.	Uninoculated.
12	Patients in Imperial Yeomanry Hospitals.	No data.	South Africa.	1900–1901	Dr A Elliot and Dr. J. W. Washbourn.	*The Lancet*, Jan. 18, 1902.	—	—
13	Patients in a Variety of Military Hospitals.	"	South Africa.	1900–1901	Dr. R. W. Dodgson.	Official report.	—	—
14	Staff of the Portland Hospital.	A few weeks.	South Africa.	Part of 1900.	Dr. Tooth.	*Brit. Med. Jour.*, Mar. 16, 1901.	28	13
15	Staff of Imperial Yeomanry Hospital, Deelfontein.	No data.	South Africa.	No data.	Dr. Elliot and Dr. Washbourn.	*The Lancet*, Jan. 18, 1902.	59	25
16	Staff of Imperial Yeomanry Hospital, Pretoria.	In most cases a few weeks.	South Africa.	Aug., 1900, to Mar., 1901.	Dr. Dodgson.	Official report.	32	72
17	No. 8 No. 9 No. 10 } General Hospitals (staffs of).	A few weeks.	Bloemfontein, South Africa.	April to Aug., 1900.	" " "	" " "	21 87 0 } 108	110 47 119 } 276
18	Staff of Second Section, Scottish Red Cross Hospital.	A few weeks.	South Africa.	Part of 1900,	Colonel Cayley, I.M.S.	*Brit. Med. Jour.*, Jan. 12, 1901.	70	12
19	Fifth Battalion, Manchester Regiment.	A few weeks.	Winburg, South Africa.	July, 1901, to Feb., 1902.	Lieutenant J. W. West, R.A.M.C.	*Brit. Med. Jour.* and *The Lancet*, April 5, 1902, reported by Prof. Wright	200	517
20	City Imperial Volunteers.	Probably a few weeks.	South Africa.	1900	Surgeon-Major R. R. Sleman, C.I.V.	Privately communicated.	700	494
21	Patients in Richmond Asylum, Dublin.	No interval.	Dublin.	Sept., 1900, to Dec., 1900.	Mr. H. M. Cullinan.	*Brit. Med. Jour.*, Oct. 26, 1901; reported by Prof. Wright.	3[illegible]9	298
22	Officers invalided from South Africa (first series).	A few weeks.	South Africa.	No data.	Colonel A. Crombie, I.M.S.	*The Lancet*, May 3, 1902	112	109
23	Officers invalided from South Africa (second series).	In most cases only a few weeks.	South Africa.	No data.	Colonel A. Crombie, I.M.S.	*The Lancet*, Aug. 16, 1902.	102	85

Number of Cases of Typhoid Fever.		Percentage Incidence of the. Disease.		Number of Deaths from Typhoid Fever.		Percentage Death-rate for Typhoid Fever.		Case Mortality—*i.e.*, Proportion of Deaths to Cases.		Supplementary Facts.
In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	
47	301	—	—	4	26	—	—	1 in 11·9	1 in 11·8	—
764	3,374	—	—	63	510	—	—	1 in 12	1 in 6·6	—
7	3	25	23	0	1	0	7	0 in 7	1 in 3	Five of the inoculated had very light attacks.
4	4	6·8	16	0	0	—	—	0 in 4	0 in 4	—
3	7	9·3	9·7	0	0	—	—	0 in 3	0 in 7	—
5 11 } 16 —	44 13 } 93 36	14·8	33·5	0 1 } 1 —	8 2 } 16 6	0·9	5·8	1 in 16	1 in 5·8	The period of observation covers the period of "the great epidemic."
2	4	2·8	33·3	1	1	1·4	8·3	1 2	1 in 4	There was no enteric in the first section, which consisted of 77 doubly inoculated and two uninoculated persons. The third section, which consisted of 20 inoculated persons, also remained free.
3	23	1·5	4·2	0	7	0	1·3	0 in 3	1 in 3·3	The three inoculated had very mild attacks. Some of the inoculated were not yet out of danger at the date of rendering the report.
60	39	8·5	7·9	9	11	1·3	2·2	in 6·7	1 in 3·5	—
6	30	1·8	10·0	1	4	0·3	1·3	1 in	1 in 7·5	The period of observation closed with the end of the epidemic.
3	24	28·5	22·0	—	—	—	—	—	—	—
34	28	33·3	32·9	—	—	—	—	—	—	The proportion of typhoid convalescents among the inoculated under 26 years of age was 1 in 3; among the uninoculated, 2 in 3; among 13 twice inoculated, 1 in 1·3; among 89 once inoculated, 1 in 3·7.

Serial Number.	Group which was under Observation.	Interval between Inoculation and Commencement of Period of Exposure.	Place where Group was exposed to Infection.	Period of Observation covered by the Statistics.	Authority for the Statistics.	Reference to published Literature or other Source of Information.	Number of inoculated and uninoculated in the Group.	
							Inoculated.	Uninoculated.
24	7th Hussars, South Africa.	A few weeks.	South Africa.	Dec. 20, 1901, to June 20, 1902.	Captain W. A. Ward, R.A.M.C.	Official report.	307	244
25	British Army in India, 1901.	1–2 years.	India.	Jan. to Dec., 1901.	Official returns.	*Brit. Med. Jour.*, Oct. 10, 1903.	4,883	55,955
26	Lord Methuen's Column, Modder River.	No data.	South Africa.	Dec., 1899, to Mar., 1900.	Surg.-Gen. Townsend, C.B.	*Ibid.*	2,535	10,981
27	No. 7, General Hospital, Natal.	Under 1 year.	Estcourt, Natal.	April to Aug. 1900.	Dr. Watkins-Pitchford.	Paper read before South African Medical Association	—	—

CRITICAL COMMENTARY ON THE STATISTICAL RECORDS INCLUDED IN THE SYNOPTICAL TABLE ABOVE.

The data[1] which are available for the evaluation of the statistical records included in the synoptical table above are set forth below under the following headings :—

A. The system of enumeration adopted ;

B. The comparability of the inoculated and uninoculated groups ;

C. The trustworthiness of the diagnosis of typhoid fever arrived at ;

D. The trustworthiness of the classification of the sick as inoculated and uninoculated respectively ;

E. The question as to how far the recorded case-mortality correctly represents the case-mortality of the inoculated and uninoculated respectively.

F. The question as to how far the incidence and death-rate is affected by the inclusion, among the inoculated, of cases infected before protection was established ;

G. The light thrown on the question of the duration of protection afforded ; and

H. The general accuracy of the record.

[1] These data are set forth more fully in Table II., appended to my former paper in the *Lancet*.

Number of Cases of Typhoid Fever.		Percentage Incidence of the Disease.		Number of Deaths from Typhoid Fever.		Percentage Death-rate for Typhoid Fever.		Case Mortality—*i.e.*, Proportion of Deaths to Cases.		Supplementary Facts.
In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	In inoculated.	In uninoculated.	
9	20	2·9	8·2	0	3	0	1·2	0 in 9	1 in 6·7	The incidence in 73 once inoculated was 4·1 per cent.; in 234 twice inoculated 2·3 per cent.
32	744	0·66	1·33	3	199	0·06	0·36	—	—	—
26	257	1·0	2·3	—	—	—	—	—	—	—
137	1,017	—	—	3	58	—	—	1 in 46	1 in 12	Percentage of mild cases 46 in inoculated, 23·99 in uninoculated: severe cases in inoculated 19·7 per cent., in uninoculated 21·92.

No. 1.—A. Nominal rolls were made of the inoculated. The number of uninoculated was calculated by subtraction of the inoculated from the total staff. B. There was no difference between inoculated and uninoculated in respect of age or period of exposure to infection; but the inoculated were in the course of their duties (nursing, laundry work) more exposed to risk than the others. Hence the circumstances in this case were more favourable to the uninoculated. C. The diagnosis was made by the medical officers of the asylum in the midst of a severe epidemic of typhoid, so that the risk of errors is very small. D. There was no chance of either inoculated or uninoculated patients being included in the wrong group. G. An agglutinating reaction was obtained two years afterwards in four out of nine inoculated cases.

No. 2.—A. Nominal rolls were made of the inoculated, and the uninoculated were calculated by subtraction of these from the average yearly strength of the unit. Some inaccuracy thus results, which may tell in favour of either group. B. The inoculated would on the average be younger than the uninoculated, as special efforts were made to inoculate the younger men. Otherwise the conditions were the same. C. The diagnosis was made by the Army Medical Officers. The existence of malaria and other tropical fevers would introduce

some risk of error. Such errors would affect both groups equally. D. Records of inoculations were entered on the Medical History-Sheets, and it is possible some errors may have crept in, in either direction, but these would hardly affect the general result. E. Some very slight attacks of typhoid may have passed unrecognised. The facts recorded under the next heading (F) must also be taken into consideration, as the case-mortality may be unfavourably influenced when patients are inoculated in the incubation-period of the disease. F. Five cases (two fatal) occurred among the inoculated at Lucknow within nineteen days after inoculation ; and one case at Agra within eight days after inoculation. By the occurrence of these cases the incidence-rate among the inoculated was increased from 0·84 per cent. to 0·98, and the death-rate from 0·15 to 0·2.

No. 3.—A. As in the last case. B.—E. The remarks made in connection with No. 2 apply here.

No. 4.—A. The method of enumeration is not on record. B. The conditions of the two groups may be assumed to be identical, as the inoculated and uninoculated are included in many different military units. C. The diagnosis was made by Army Medical Officers and Civil Surgeons, working under the strain of war in a beleagured town. D. As to the classification, *see* under No. 13, p. 60. H. Of two deaths recorded to have occurred among inoculated officers, one was due to a casualty in the field. The other officer had been inoculated with a serum, not with anti-typhoid vaccine. Among the uninoculated officers a minimum of ten deaths occurred, instead of five as recorded. When due allowance is made for these errors, the case-mortality of the inoculated works out distinctly lower than that of the uninoculated.

No. 5.—A. The number of inoculated is represented to be the number inoculated since rendering previous returns ; the number of uninoculated is calculated by subtracting these from the total average strength of the garrison. The statistical results are rendered unduly favourable to the uninoculated by the fact that among these are included in the records 234 men (11th Hussars) inoculated in India (1889) and a balance of 463 men inoculated in Egypt (1889). There were no cases of typhoid in either of these groups. F. The only case

of typhoid fever among the inoculated was a fatal case in a man inoculated on December 8, 1899, and admitted to hospital January 9, 1900. If it had not been for this case, the incidence on the inoculated, numbering probably over 1,000, would have been nil. G. The fact that no case of typhoid occurred among those inoculated in the previous year seems to indicate a persistence of the protection. H. The number of the inoculated in the West Kent Regiment (228), given as representing men newly inoculated, is identical with the number from that regiment reported as inoculated in the previous year. This suggests a mistake in the entry.

No. 6.—A. The number of inoculated was obtained by adding together the numbers reported from the separate stations; it thus happened that in some cases men were counted twice over, being returned from two places. It is estimated that the total of the inoculated may be wrong by at most "some hundreds." The numbers of the uninoculated are in some cases actuals, in others they are obtained by subtraction of inoculated from the average yearly strength of units. B.—D. The conditions for the most part are as under No. 2. In the case of some at least of the 2,246 men inoculated in 1900 the period of exposure after inoculation must have been less than a complete year. F. In the case of three of the inoculated who contracted typhoid, the attacks supervened respectively 13, 15 and 24 days after inoculation. The first of these cases ended fatally. If these had been deducted, the incidence and death-rate among the inoculated would have been slightly reduced—the case-mortality from 1 in 6·5 to 1 in 7. G. Of the 52 cases of typhoid which occurred in the inoculated in 1900, 21 occurred among the 2,250 inoculated in 1900, and 31 among the 3,750 inoculated in 1889. These figures suggest that the protection persists for more than a year.

No. 7.—A and D. No data are available as to procedure adopted for purpose of classifying the patients into inoculated and uninoculated. C. The case-mortality (1 : 53) attributed to the uninoculated is suspiciously low for typhoid fever. The case-mortality of the inoculated (1 : 15) corresponds with that found elsewhere in South Africa. H. The total typhoid case-mortality of the hospital in which Dr. Melville served

in the months which are covered by his period of observation was 1 in 5·4, not 1 in 40 as would appear from Dr. Melville's figures. (Major S. Westcott, letter to *British Medical Journal,* July 30, 1901.)

No. 8.—A. and D. The classification of the sick into inoculated and uninoculated was controlled by a careful triple check. C. Major C. Birt, R.A.M.C., confirmed the diagnosis in all fatal cases by necropsy, and all cases of doubtful diagnosis were omitted from the records.

Nos. 9 to 12.—A. and D. No data available as to procedure adopted for the purpose of classifying the patients into inoculated and uninoculated. C. Diagnosis made by the physicians attached to the hospitals.

No. 13.—A. and D. In most cases entries were made in the case of the inoculated, and blanks were left in the records of the uninoculated. Cases transferred from one hospital to another are omitted from the statistics. C. As in No. 2. D. Errors of classification may have occurred owing to statements made in misapprehension by patients who had been vaccinated against small-pox on the transports, or to erroneous statements made by patients who desired for the purpose of avoiding inoculation to represent themselves as inoculated. E. Both officers and men are included in the statistics, the former having, perhaps, a better chance of recovery; but as both classes occur in each group, there is no resulting source of error. H. Dr. Dodgson's report which sets forth the figures collected from the Military Hospitals affords evidence of conscientious compilation and the exercise of a critical spirit in dealing with statistical records which were often vitiated by serious errors.

Nos. 14, 15, and 16.—A. Presumably nominal rolls. B. No data as to age and period of exposure in the two groups C. The diagnoses were made by the physicians attached to the hospital.

No. 17.—A. Nominal rolls. B. The conditions of the three hospitals whose inoculated and uninoculated staff are here compared were similar; the incidence rate among the uninoculated members was in No. 8, 40 per cent., in No. 9, 28 per cent., and in No. 10, 30 per cent. F. Four cases (none fatal) occurred between the third and the fourth week after inoculation.

No. 18.—A. The uninoculated in the second section probably numbered less than 12. B. As in 14–16 supra. D. As in No. 1.

No. 19.—A. Presumably nominal rolls. B. Conditions the same. C. and D. Diagnosis was in each case made by Lieut. J. W. Wells, who remained with the regiment after carrying out the inoculations.

No. 20.—No data beyond those included in the Table.

No 21.—A. Nominal rolls were made of the patients as they were inoculated in a series of separate sittings. From these and from a list of the cases and dates of attack the average strength of the inoculated and the uninoculated was calculated, for the period covered by the epidemic. B. No data as to age. The average age of the nurses (uninoculated) may probably have been less than that of the patients. The external conditions of both groups were similar, but the nurses (uninoculated), though living among the insane patients, would avoid risks incurred by the latter. Reckoned by the incidence of cases among the uninoculated, the risk of coming in contact with infection was greater after the date at which the number of inoculated reached the number taken as the average strength than it was before this date. C. and D. No source of error. F. Of a total of 6 cases of typhoid fever which developed in the inoculated, 5 occurred within twelve days of inoculation ; 1 of these ended fatally. If these cases had been deducted, the incidence on the inoculated group would have been 30- (instead of 6-) fold less than on the uninoculated.

No. 22.—A. The inoculated and the uninoculated represent two chance groups marked off by the accident of invaliding from the large bodies of inoculated and uninoculated officers who saw service in South Africa. The statistics are vitiated in favour of the uninoculated, for seeing that the typhoid case-mortality of the inoculated is, as is brought out by the sum total of the available statistics, 50 per cent. lower in the inoculated, more of these would survive to return home. B. The uninoculated were probably older and by reason of their age less susceptible (*see* next paragraph). C. and D The classification of the patients as convalescents from typhoid depended upon the reports they rendered of the diagnoses arrived at in South Africa. F. One twice-inoculated officer contracted typhoid within a month of landing in South Africa.

G. A table in Colonel Crombie's report suggests that the effect of inoculation diminishes after the expiration of six months.

No. 23.—A. As in No. 22. B. The average age of the inoculated was 25·4 years, that of the uninoculated 30·6. C. and D. As in No. 22.

No. 24.—A. Nominal rolls. C. and D. The classification into inoculated and uninoculated, and the facts with regard to incidence and case-mortality were very carefully controlled by Captain W. A. Ward, R.A.M.C., who conducted the inoculations and remained in medical charge of the regiment.

No. 25.—A. The number of the inoculated and uninoculated corresponds in each case with the average strength of the group determined by taking into account the number of days during which each soldier was under observation. B. to E. As in No. 2. F. No data. G. The statistics here in question refer almost exclusively to men inoculated in 1898, 1899, and 1900. So far as they apply to men inoculated in 1900, they confirm the statistics of that year, which showed that the protection conferred by inoculation persisted during the second term of twelve months. So far as the statistics of 1901 refer to a residue of the 4,000 men inoculated in India in 1898–1899 they suggest that the protective effect of inoculation is maintained for a minimum of three years.

No. 26.—No data beyond those included in the Table.

No. 27.—No data beyond those included in the Table.

GENERAL CONCLUSIONS WHICH EMERGE FROM THE SUM TOTAL OF STATISTICAL RECORDS.

It will be well to make clear before proceeding to summarise the teaching of the statistical materials embodied in the table above that the inoculations whose results are here recorded represent inoculations undertaken for the most part in a very hurried manner, under very difficult external conditions, with imperfectly standardised vaccines, and particularly in the case of the South African inoculations, by agents who were absolutely without scientific training.[1] Further the results here recorded were obtained for the most part by the inoculation

[1] This defect of training was exhibited in the fact that great liberties were taken in the matter of the dose inoculated, further in the fact that the essential element of the vaccine, the bacterial deposit, was often left at the bottom of the bottle, the supernatant fluid being alone employed.

of a single large dose of vaccine. If, as will be plain from the analysis of the results which is given below, inoculations conducted under all these disadvantages have none the less furnished clear evidence of the utility of the process, and if, as will be manifest, the process has—in spite of the imperfect manner in which it has been carried out—already effected a great saving in life, it is perhaps not over-sanguine to look forward to the achievement of a very much greater protective effect when provision shall have been made for carrying on the inoculations in a manner more in accordance with the demands of a scientific system.

For the moment, however, we are concerned, not with the future, but with what has already been achieved.

Effect which has been exerted by anti-typhoid inoculation on the incidence of the disease.

A study of the statistical material will show that in every case, except that of the City Imperial Volunteers (where the statistical materials at my disposal consist only of the bald figures incorporated in the table), and in the case of the staffs of the Portland Hospital and Imperial Yeomanry Branch Hospital at Pretoria (where we are dealing with very small figures), the incidence of typhoid fever was diminished by at least one-half in the inoculated. In certain cases, notably in those to which the statistics designated by the serial numbers 3, 4, 5, 18, and 21 apply, a greater reduction in the incidence of typhoid fever was achieved—a reduction varying from a 6-fold to a 28-fold reduction.

Effect which has been exerted by anti-typhoid inoculation on the case-mortality of the disease.—Superadded to the diminished incidence of the disease there is a striking diminution of case-mortality, as is attested by the extensive statistical material collected by Dr. R. W. Dodgson (Table I., No. 13), from various Military Hospitals, and more particularly by the accurate and extensive figures obtained at Harrismith under the zealous personal supervision of Major C. Birt, R.A.M.C. (Table I., No. 8), and in the Civil Hospitals (Tables I. and II., Nos. 9, 10, 11, 12, 14) in South Africa. In the aggregate the proportion of deaths to cases among the inoculated has been rather less than half that among the uninoculated.

Taking the figures in the Table exactly as they stand, it

will be found that 1,758 inoculated patients furnished 142 deaths (8·0 per cent.), while 10,980 uninoculated patients furnished 1,800 deaths (16·6 per cent.).

Effect which has been exerted by anti-typhoid inoculation on the death-rate from the disease.—The combined effect of the diminished incidence and diminished case-mortality manifests itself in the diminished death-rate from typhoid fever among the inoculated. The minimum reduction of death-rate chronicled in the table is the twofold diminution in the case of the City Imperial Volunteers. As will be seen, the reduction of death-rate in case of the inoculated has often exceeded, and has seldom fallen below, a four-fold reduction.

Duration of the protection which is conferred by anti-typhoid inoculation.—The only definite statistical evidence which is available for the decision of the question as to the duration of the protective effect exerted by anti-typhoid inoculation is to be found in the statistical returns of the year 1900, relating to the British Garrisons in India and Egypt respectively. (Synoptical Table, Nos. 6 and 5.) This evidence, so far as it goes, points to the persistence of the protective effect during the second year after inoculation.

The statistics relating to the British garrison in India (Synoptical Table, No. 25) for the year 1901 make it probable that the effect of the inoculation persists during the third year.

Attention may be directed in this connection to two series of laboratory observations which afford a certain amount of support to the conclusions just arrived at:—

(*a*) It is not infrequent to find an agglutinating power in the blood of inoculated persons as long as two years after the inoculation of anti-typhoid vaccine.

(*b*) Von Dungern's experiments [1] in connection with rabbits inoculated with crab's blood have shown that even after the specific antitropic substances which have been produced by inoculation have disappeared from their blood, the inoculated animals retain a power of responding more rapidly and more effectively to a renewal of the vaccinating stimulus.

[1] *Loc. cit.*

APPENDIX I.

DETAILED DESCRIPTION OF THE PROCEDURE EMPLOYED FOR PREPARING AND STANDARDISING ANTI-TYPHOID VACCINE.

CULTIVATION FLASKS.

For the purpose of cultivation flasks of the pattern shown in Fig. 1 are employed. These flasks are, as will be seen, furnished with a lateral tube, to which is fitted a piece of perfectly sound vulcanised rubber pressure tubing. The orifice of this last is blocked with a piece of glass rod, and is fitted with a pinch cock (*see* figure) in such a manner that the distal end of the tube remains free from fluid.

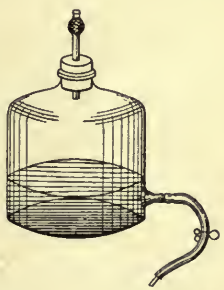


Fig. 1.—Cultivation flask.

The advantages which are associated with the employment of cultivation flasks fitted in this manner are the following :—The flasks can easily and rapidly be inoculated by puncturing through the pressure tubing with the needle of a syringe which has been filled with a pure culture of typhoid. In doing this, as a precaution against the introduction of contaminations, the outside of the tubing, at the point where it is to be punctured, is first sterilised by the application of a piece of glass rod which has been raised to a dull red heat. After withdrawing the needle the minute puncture hole can readily be sealed up by reapplying the heated glass. Further advantages which are associated with the use of these flasks are (*a*) the possibility of withdrawing samples at any time with a view to controlling the purity of the cultures, and (*b*) the possibility of transferring, without exposure to the air, the contents of the cultivation flasks to the larger jars (Fig. 2) employed in the processes of preparation which are described below.

CULTIVATION MEDIUM AND PERIOD DURING WHICH THE CULTURES ARE INCUBATED.

The flasks, after having been inoculated, are transferred to an incubator, which is kept at blood temperature, ordinary 1 per cent. peptone broth, accurately neutralized and disposed in a shallow layer, furnishes the best cultivation medium. The cultivation is continued for a maximum of 48 hours.

MODE OF FITTING UP THE MIXING JARS IN WHICH THE CULTIVATIONS ARE STERILISED BY HEAT.

After the purity of each flask has been ascertained by culture, the contents of the cultivation flasks are transferred to the larger jars (Fig. 2). This transference is undertaken first with a view to mixing together the contents of a series of cultivation flasks, so as afterwards to standardise them in bulk ; secondly, it is undertaken with a view to eliminating the possibility of the vaccine becoming spontaneously reinoculated with living bacilli after it has been exposed to heat. This possibility would exist if there were to be found on the sides or neck of the flask, when it is transferred to the water bath, any typhoid bacilli which had been rendered by desiccation less sensitive than they would otherwise be to the heat which is there brought to bear on them.

Before describing the process of transferring the typhoid cultures from one flask to another, and the subsequent processes of heating and mixing, it will be necessary first briefly to describe the manner in which the larger "mixing" jars are fitted up.

The lateral tubes are fitted with vulcanised rubber pressure tubing, which is blocked in each case with glass rod. A clamp is further placed on each tube. Through the lowest of the lateral tubes the contents of the cultivation flasks are introduced into the mixing jar. The upper tube is appropriated to a paraffin thermometer which is employed for the purpose of notifying the point at which the internal temperature of the typhoid culture has reached

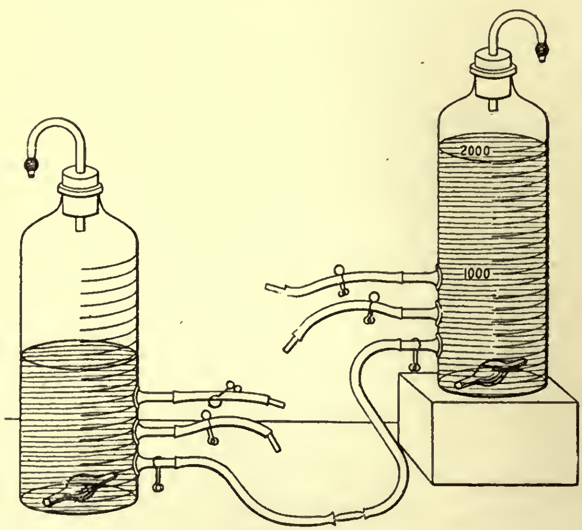


Fig. 2.—Large jars connected together for the purpose of mixing the vaccine. Paraffin thermometers which have sunk are shown at the bottom of each mixing jar.

60° C. The principle and the mode of using these thermometers require a word or two of explanation.

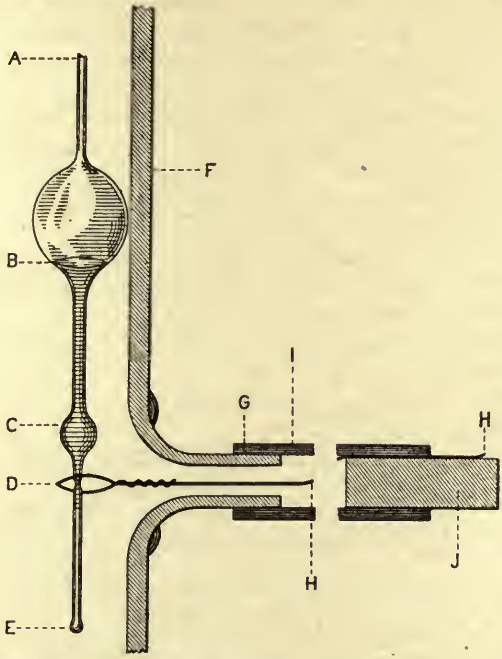


Fig. 3.—*Paraffin thermometer in position against inner wall of mixing jar.* A, *Upper end of thermometer, left open;* B, *bulb;* C, *fusiform enlargement filled in with paraffin of the appropriate melting point;* D, *constricted portion of the stem engaged in wire snare;* E, *lower sealed end of thermometer;* F, *wall of mixing jar;* G, *upper lateral tube of mixing jar;* H, *distal end of wire snare engaged between the rubber tube* (I) *and the glass rod* (J) *which blocks the tube.*

CONSTRUCTION AND MODE OF EMPLOYING THE PARAFFIN THERMOMETERS.

The construction of the paraffin thermometer will readily be understood from a consideration of Fig. 3 which represents the thermometer in position in the mixing jar. It will be seen that the thermometer consists essentially of a glass bulb or float which is left open at the top. This glass bulb runs out below into a hollow glass stem, which is filled in with paraffin through its lower end. When the thermometer is brought into use the bulb floats on the surface of the fluid. The paraffin chamber, acting as a sinker, causes the thermometer to stand up in the fluid somewhat like a fisherman's float. When the temperature of the surrounding fluid rises to 60° C. (or to such other point as corresponds to the melting point of the particular paraffin which is employed), the melting paraffin is pressed upwards against the upper part of

the paraffin chamber by the pressure of the fluid. Owing, however, to the fusiform shape which is given to this chamber (Fig. 3, C), the fluid cannot pass up into the stem until the paraffin actually melts. As soon as this occurs the fluid enters the upper bulb or float and the thermometer sinks to the bottom.

These thermometers having been devised, the only difficulty which presented itself was that of so disposing matters as to allow of the thermometer being sterilised *in situ*. This object is effected by sealing up the lower end of the thermometer (Fig. 3, E) so as to prevent the paraffin escaping during the process of sterilisation. The further problem of snapping off the sealed end of the thermometer without opening up the sterilised jar is satisfactorily solved by the following device.

When the jar is being fitted up, previous to sterilisation, a noose of fine brass wire is passed in through the rubber tube which is connected with the uppermost lateral tube of the mixing jar.

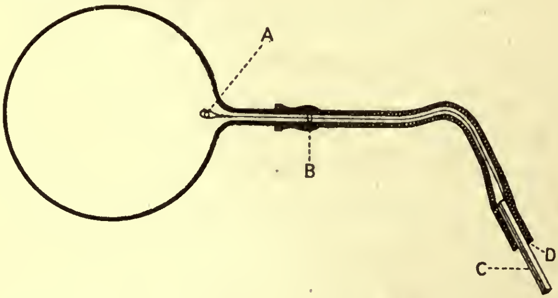


Fig. 4.—*Transverse section of mixing jar at the level of the uppermost lateral tube, showing method of fixing the paraffin thermometer.* A, *Noose of brass wire engaging the constricted part of the stem of the thermometer;* B, *end of lateral tube;* C, *glass rod plugging the end of the rubber tube;* D, *free end of the wire clamped in position by the rod* C.

This noose is brought up to the neck of the jar, and the sealed capillary end of the thermometer is engaged in it in such a manner that the noose fits into a constriction shown in the diagram (Fig. 3, D). The noose is now drawn gently home until the thermometer becomes fixed, opposite the orifice of the lateral tube. It only remains to fasten the free extremity of the wire. This is effected by inserting a piece of tightly-fitting glass rod into the end of the tube in such a manner as to engage the wire between the rod and the wall of the tube (Figs. 3 and 4). This done, a rubber bung, which is perforated by a recurved glass tube plugged with cotton wool, is inserted into the neck of the jar, and is tied in tightly. The jar is now ready for autoclaving.

METHOD OF MIXING TOGETHER THE CONTENTS OF A SERIES OF CULTIVATION FLASKS AND OF KILLING THE CULTURE.

When the jar has been autoclaved, and when the bung has afterwards been duly luted with sterilised paraffin, the subsequent procedure is as follows :—A cultivation flask is taken in hand, the end of the attached rubber tubing is sterilised in the flame, and the glass rod which blocks the orifice is

withdrawn in an aseptic manner. In a similar manner the glass rod which blocks the lowest of the three lateral tubes of the mixing jar is withdrawn. This done, a connection is, by means of a sterilised glass union, established between the two tubes, the clamps are withdrawn, and the fluid passes out from the cultivation flask into the mixing jar. As soon as a certain amount of fluid has entered the mixing jar, and at any rate before the fluid has reached the level of the uppermost lateral tube, the pressure tubing which is attached to this side tube is gently pulled. This causes the wire noose which is round the capillary end of the paraffin thermometer to snap this off by a guillotine action. The released thermometer then drops into the fluid, ready for use. The brass wire which has fulfilled its office is now grasped with a forceps, and is withdrawn from the tube.

When the contents of a cultivation flask have passed into the mixing jar, the clamp belonging to the mixing jar is replaced upon the rubber tube at a point proximal to the glass nozzle which connects the two parts. The needle of a syringe which has been filled with undiluted carbolic acid is then inserted into the lumen of the tube at a point between the clamp and the glass nozzle which was referred to above. The syringeful of carbolic acid is then driven through the tube into the cultivation flask. This done, the cultivation flask may be disconnected without risk. The next cultivation flask in the series is now taken in hand in the same manner. When the process of filling in the mixing jar has been completed the side tubes are blocked with pieces of sterilised glass rod. These are luted with rubber solution.

The next proceeding is to transfer the mixing jar or jars to the water bath. This is to be done without delay, and the jars are to be carried in such a manner as to avoid all splashing up of the culture on to the sides and neck of the jar. The water bath must be deep enough to allow of the water coming well over the shoulder of the mixing jar. This last is not to be filled within 2 inches of this level. If the jar, when it is introduced into the water bath, is found to be buoyant, it is weighted down by passing a heavy leaden collar round its neck. The jars having been securely placed in position, heat is applied to the water bath, and the condition of the thermometers in the separate jars is noted from time to time. This is best done by lowering a piece of ordinary looking-glass below the surface of the water in the water bath. The gas is turned out as soon as the paraffin thermometer has sunk. The jar of vaccine remains in the hot water for another 10 to 15 minutes.

The next step is to mix the contents of the whole series of mixing jars so as afterwards to obtain for standardisation a representative sample of the whole brew of vaccine. The mixing is effected by connecting up the mixing jars with each other by means of their rubber tubes (Fig. 2). These rubber tubes are of such length as to allow of any one of the jars being freely raised above or lowered below the level of its companion jars. By these means complete mixture of the contents is readily effected. In cases where the mixing jars are so full as to place a difficulty in the way of the satisfactory mixture of their contents, an additional empty sterilised mixing jar is introduced into the circuit. Mixture having been duly effected, samples of the vaccine are withdrawn in the manner described above in connection with the

control of the purity of the contents of the cultivation flasks. The sterility of the samples of vaccine is of course tested by introducing them both into tubes of broth and into tubes of nutrient agar.

After this has been done further samples are drawn off for the purpose of determining the strength of the vaccine. An addition of antiseptic is now made to the vaccine with a view to preserving it against risk of subsequent contamination. An addition of one-tenth of its bulk of a 5 per cent. solution of lysol or carbolic answers this purpose very well. A simple method of making this addition is to take the required quantity of water and to sterilise it in a Kitasato flask fitted with a length of vulcanised pressure tubing. After sterilisation the appropriate quantity of carbolic acid is introduced into this flask by puncturing through the pressure tubing. The Kitasato flask is now connected up with the series of mixing jars which contain the vaccine, and the antiseptic is thoroughly mixed up with the vaccine. It is inexpedient to add undiluted carbolic acid directly to the vaccine, inasmuch as it causes the bacteria to run together into large masses.

STANDARDISATION OF THE VACCINE.

As has been indicated when this subject matter was under consideration in Chapter III. above, the vaccine can be standardised in a satisfactory manner by the enumeration of the typhoid bacilli contained in the unit of volume.

This enumeration can in the case of a 24—48 hour culture of the bacillus typhosus be made in an accurate and expeditious manner under the microscope by the method described by me in the *Lancet*, July 5, 1902.

The figure below will illustrate the method. It will be manifest that in the case where we are dealing with a mixture of an aliquot volume of normal[1] blood (containing 5,000,000,000 red blood corpuscles per cubic centimetre) with an equal volume of a bacterial culture, the number of micro-organisms and red blood corpuscles in a film prepared with such a mixture will stand one to the other in the ratio which corresponds to the respective numbers of red corpuscles and bacilli in the unit of volume.

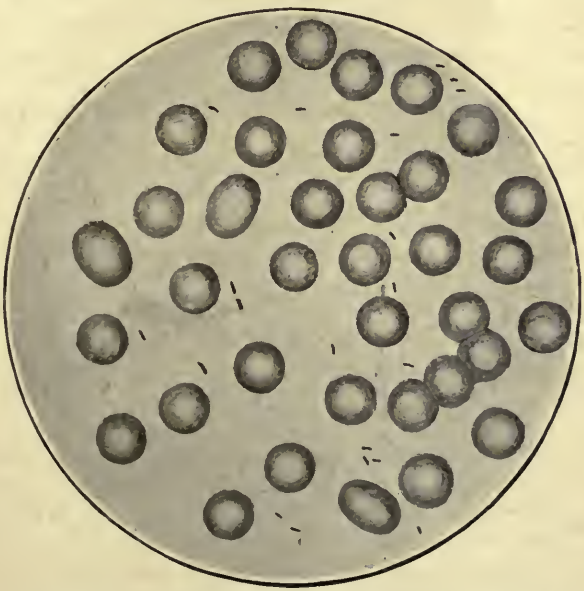
The mixture of the blood and culture in definite proportions is made as follows.[2] We take in hand a piece of glass tubing, heat it and draw it out into a capillary stem of convenient size. We provide ourselves with a glass-writing pencil and a rubber teat which will fit tightly upon the upper end of our tube. Having placed a mark upon the capillary stem at a convenient distance from its orifice, and having by means of the teat established a negative pressure in the interior of the tube, we draw up in succession into the capillary stem (*a*) one volume of blood (obtained from a prick in the finger) such as will fill the capillary stem up to the fiduciary mark ; (*b*) an air bubble to serve as an index ; (*c*) a volume of the vaccine, (*d*) and further three volumes of a physiological salt solution ; or, in lieu of these last, as may be required, one, two, or three further volumes of the vaccine ; taking care to complete in each case to five

[1] Where only blood which exerts an agglutinating action on the typhoid bacillus is available, the difficulty which arises may be circumvented by heating the culture of typhoid to the point at which the agglutinable substance is destroyed.

[2] Examples of the employment of the different mixtures here in question will be found tabulated in Table I., appended to my original paper.

volumes. Having expelled the contents of the tube on to a slide, and having effected thorough mixture by drawing up the fluid into the capillary pipette and blowing it out several times in succession, we transfer a drop of the mixture to a microscopic slide[1] and spread it out by bringing down upon it the edge of another slide and drawing this along. The thickness of the film is regulated by adjusting the angle between the slides, widening this angle when a thicker film is to be left behind, and narrowing it when a thinner film is required The film is then fixed and stained, most conveniently, perhaps, by the stain described by my sometime colleague, Major W. B. Leishman, R.A.M.C. The bacteria and red blood corpuscles are now separately enumerated on a series of fields chosen at random from different parts of the preparation. Owing to the spacing out which is secured by the five-fold dilution of the blood this enumeration can, if necessary, be carried out without restricting or subdividing the field of the microscope. It, however, facilitates matters if we restrict the field by the employment of a high-power eye-piece and subdivide it by inscribing a cross by means of a glass writing pencil upon a cover-glass and dropping this into the eye-piece. The necessary calculations can be most easily carried out by the help of a slide rule.

The method of calculation will be rendered intelligible on considering the figure below.



Film preparation made from a mixture of a measured volume of normal blood with a measured volume of typhoid culture.

[1] The slide may with advantage have been prepared immediately before use by pouring alcohol upon it and burning this off. The same object can be achieved by boiling the slide in strong caustic soda.

We have here represented a typical field of such a film preparation as has been described above. It will be found that there are here contained in this field of view 36 red blood corpuscles and 20 typhoid bacilli. Making the assumption that we are here dealing with a normal blood and as already postulated with an average field, the calculation we make is as follows :—

Number of red blood corpuscles in average field : number of bacilli in average field :: number of red blood corpuscles in the unit of volume : number of bacilli in the unit of volume.

36 : 20 :: 5,000,000,000 number of bacilli in the cubic centimetre of culture : answer :—The culture contains circ. 2,800,000,000 typhoid bacilli in the cubic centimetre.

In the case where, in lieu of 1 volume, 2, 3, or 4 volumes of culture have been mixed with one volume of blood the number of bacilli in the cubic centimetre of culture will of course correspond to the quotient obtained by dividing the result arrived at in the above calculation by, as the case may be, 2, 3, or 4.

Bottling the Vaccine.

In order to reduce to a minimum the possibility of aerial contamination during the operation of bottling, the vaccine is by the following procedure run from the large jars directly into bottles without being at any time exposed to the air.

In the first place a number of small bottles, of a shape and capacity best suited to the requirements of the case, are plugged with cotton-wool and autoclaved.

When the autoclaved bottles are cool the cotton-wool plugs are replaced under antiseptic precautions by strong rubber caps, which have been soaked in a saturated solution of perchloride of mercury, and which have been specially made to fit the bottles. The use of these rubber caps, in preference to any form of cork or stopper, presents many advantages, chief among them being the fact that they admit of all fluids being introduced into, or withdrawn from, the bottle without further opening than that made by the introduction of a hypodermic needle—an obvious gain when we consider the danger of contamination which occurs every time a bottle is corked or uncorked.

A sufficient number of bottles having been prepared in this manner, one of the mixing jars is now taken in hand. The lowest of the lateral tubes is now connected by means of a glass nozzle with a length of sterilised rubber tubing, terminating in a T tube fitted up by means of further lengths of rubber tubing with two hypodermic needles. To the plugged air tube which passes through the top of the missing jar is now attached an ordinary hand bellows, such as belongs to any spray-producing apparatus. This is done with a view to accelerating by air pressure the flow of the vaccine as it passes out through the hypodermic needles. In order to prevent the paraffin with which the rubber bung is luted from yielding to air pressure when this comes to be applied, it is well to coat over every part of the bung with a layer of collodion, which should be allowed to set before pressure is applied. The hypodermic needles are now withdrawn from the test tubes in which they have been sterilised, and we proceed to fill in the bottles. The first operation is to get

up a slight amount of air pressure in the interior of the jar by working the bellows. This done the caps which cover the bottles are dipped in a strong solution of carbolic acid or lysol. A vent is now provided for the air, which

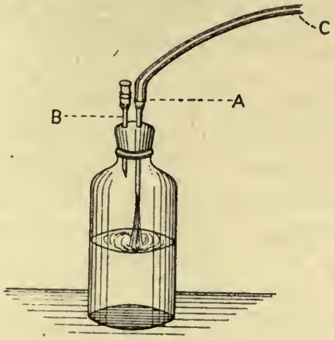


Fig. 6.—*Method of filling small bottle from the mixing jar.* A. *Hypodermic needle connected with the tube* C *leading from the mixing jar;* B. *second hypodermic needle forming an outlet for the air.*

will be displaced by the vaccine by piercing the cap of each bottle as it is dealt with by a very fine hypodermic needle, which has just been removed by means of a forceps from a vessel of oil at a temperature exceeding 100° C. (Fig. 6). The clamp on the rubber tubing which leads off from the mixing jar is now taken off, and the pressure of the fingers is substituted for it. The flow of the vaccine is now under perfect control, the hypodermic needle is introduced through the cap into the bottle, and the vaccine is then allowed to flow in (Fig. 6). In this manner any number of bottles of vaccine can be quickly filled, without the fluid ever coming in contact with the external air. The contents of the mixing jar are shaken up from time to time during the course of the operation.

When the bottles have been filled, it remains to repair the microscopic punctures in the rubber caps and to coat them with a protecting envelope of paraffin. For this purpose the solution of rubber which is sold for the purpose of repairing bicycle tyres is employed. Before applying it to the cap of the bottle. the remains of the antiseptic solution are first cleaned off by absolute alcohol, and then the rubber is freed from all traces of grease by means of ether. When the rubber solution has hardened, each bottle is further protected by coating the whole cap with paraffin, which has been previously raised to a temperature of 160° C. A paraffin with a high melting point is selected if the vaccine is to be sent to a tropical climate. In dipping the bottles into the melted paraffin it is well to immerse the whole cap, so that the paraffin may fill up the interspace between the base of the cap and the neck of the bottle.

To withdraw the vaccine from one of the bottles for inoculation it is only necessary to sterilise the cap in hot oil, or, better, in a hot antiseptic solution, and to insert a sterile syringe through the rubber cap. After the first puncture the needle is withdrawn and then reinserted and the syringe filled. The first perforation is made for the purpose of acting as an air valve to permit the entrance of air to replace the fluid withdrawn.

APPENDIX II.

LIST OF AUTHOR'S PAPERS DEALING WITH IMMUNISATION AGAINST BACTERIAL DISEASES; ANTI-TYPHOID INOCULATION; THE MEASUREMENT OF THE CONTENT OF THE BLOOD IN PROTECTIVE SUBSTANCES; AND KINDRED SUBJECTS.

Wright.—On Wooldridge's method of producing immunity against anthrax by the injection of solutions of tissue-fibrinogen.—*British Medical Journal*, September 19, 1891.

Wright and Bruce.—On Haffkine's method of vaccination against Asiatic cholera.—*British Medical Journal*, February 4, 1893.

Wright and Semple.—On the presence of typhoid bacilli in the urine of patients suffering from typhoid fever.—*Lancet*, July 27, 1895.

Wright.—On the association of serous hæmorrhages with conditions of defective blood-coagulability.—*Lancet*, September 19, 1896.

(*In this paper, which treats inter alia of the local effects produced by anti-typhoid inoculation, the details of the two first anti-typhoid inoculations conducted upon man are placed upon record.*)

Wright.—Note on the technique of serum diagnosis of acute specific fevers.—*British Medical Journal*, January 16, 1897.

Wright and Semple.—Remarks on vaccination against typhoid fever.—*British Medical Journal*, January 30, 1897.

Wright and Smith.—On the application of the serum-test to the differential diagnosis of typhoid and Malta fever.—*Lancet*, March 6, 1897.

Wright.—A further note on the technique of serum-diagnosis.—*British Medical Journal*, February 5, 1898.

Wright and Lamb.—Observations on the question of the influence which is exerted by the agglutinins in the infected organism.—*Lancet*, December 23, 1899.

Wright and Leishman.—Remarks on the results which have been obtained by the anti-typhoid inoculations, and on the methods which have been employed in the preparation of the vaccine.—*British Medical Journal*, January 20, 1900.

Wright.—On a method of measuring the bactericidal power of the blood for experimental purposes. (First communication.)—*Lancet*, December 1, 1900.

Wright (conjointly with other Members of the Indian Plague Commission).—On Haffkine's anti-plague inoculation.—*Report of Indian Plague Commission*, 1901.

Wright.—On the quantitative estimation of the bactericidal power of the blood (second communication.)—*Lancet*, March 2, 1901.

Wright.—On the changes effected by anti-typhoid inoculation in the bactericidal power of the blood with remarks on the probable significance of these changes.—*Lancet*, September 14, 1901.

Wright.—On the results obtained by anti-typhoid inoculation in the case of an epidemic of typhoid fever which occurred in the Richmond Asylum, Dublin.—*British Medical Journal*, October 26, 1901.

Wright.—Notes on the treatment of furunculosis, sycosis, and acne by the inoculation of a staphylococcus vaccine; and generally on the treatment of localised bacterial invasions by therapeutic inoculations of the corresponding bacterial vaccines.—*Lancet*, March 29, 1902.

Wright.—On some new procedures for the examination of the blood and bacterial cultures.—*Lancet*, July 5, 1892.

(*Includes a description of a method of determining under the microscope the number of micro-organisms contained in a bacterial culture.*)

Wright.—Synopsis of the results which have been obtained by anti-typhoid inoculation.—*Lancet*, September 6, 1902.

Wright and Windsor.—On the bactericidal effect exerted by human blood on certain species of pathogenic micro-organisms, and on the anti-bactericidal effects obtained by the addition to the blood in vitro of dead cultures of the micro-organisms in question.—*Journal of Hygiene*, Vol. II., No. 4, October, 1902.

Wright.—On the measurement of the bactericidal power of small samples of blood under ærobic and anærobic conditions, and on the comparative bactericidal effect of human blood drawn off and tested under these contrasted conditions.—*Proc. Royal Society*, Vol. 71, 1902.

Wright and Knapp.—Note on the causation and treatment of thrombosis occurring in connection with typhoid fever.—*Transactions of the Medico-Chirurgical Society*, Vol. 86, and *Lancet*, December 6, 1902.

Wright.—On the bacteriolytic power of the blood and on its relation to the problems of anti-typhoid inoculation and the recent work of Dr. Macfadyen.—*British Medical Journal*, April 4, 1903.

Wright.—A note on the serum reaction of tubercle with special reference to the intimate nature of agglutination reactions generally and to the therapeutic inoculation of the new tuberculin.—*Lancet*, May 9, 1903.

Wright.—A lecture on the therapeutic inoculation of bacterial vaccines and their practical exploitation in the treatment of disease.—*British Medical Journal*, May 9, 1903.

Wright.—On some further improvements in the procedures for testing and judging by the naked eye of the agglutinating and bacteriolytic effects exerted by the sera of patients suffering from, or preventively inoculated against, typhoid fever, Malta fever, and tuberculous affections.—*Lancet*, July 25, 1903.

Wright.—On the protective effect achieved by anti-typhoid inoculation as exhibited in two new statistical reports.—*British Medical Journal*, October 10, 1903.

Wright and Douglas.—An experimental investigation of the rôle of the blood-fluids in connection with phagocytosis.—*Proc. Royal Society*, Vol. 72, 1903.

Wright.—On certain new methods of blood examination with some indications of their clinical importance.—*Lancet*, January 21, 1904.

Wright.—On anti-typhoid inoculation.—*Practitioner*, January, February, and March, 1904.

(*The present volume consists of a reprint of these papers.*)

Wright and Douglas.—Further observations on the rôle of the blood fluids in connection with phagocytosis.—*Proc. Royal Society*, Vol. 73, 1904.

Wright.—On the treatment of acne, furunculosis, and sycosis by the therapeutic inoculation of a stapylococcus vaccine.—*British Medical Journal*, May 7, 1904.